CLINICOPATHOLOGICAL ANALYSIS OF OVARIAN TUMOURS AND THE ROLE OF ER, PR AND HER-2/neu IN SURFACE EPITHELIAL TUMOURS OF OVARY

DISSERTATION
SUBMITTED FOR M.D.(PATHOLOGY)
BRANCH III
APRIL 2016

THE TAMILNADU DR.M.G.R. MEDICAL UNIVERSITY
CHENNAI – TAMILNADU

CERTIFICATE

This is to certify that this dissertation titled "**CLINICOPATHOLOGICAL ANALYSIS OF OVARIAN TUMOURS AND THE ROLE OF ER, PR, HER-2/neu IN SURFACE EPITHELIAL TUMOURS OF OVARY"** is the original and bonafide work done by **DR.T. PRIYA** under the guidance of **Prof. Dr. AL. SANTHI, M.D.,** Professor & Head, Department of Pathology at the Thanjavur Medical College and Hospital, Thanjavur during the tenure of her course in M.D. Pathology from May 2013 to April 2016 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai – 600032.

Prof. Dr. AL. SHANTHI, M.D.,
Professor & Head,
Department of Pathology,
Thanjavur Medical College and Hospital,
Thanjavur

Place : Thanjavur

Date : -10-2015

Prof. Dr. SINGARAVELAN,
M.D.(Paed.), DNB (Paed.)
Dean in-charge,
Thanjavur Medical College and Hospital,
Thanjavur

Place : Thanjavur

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CLINICOPATHOLOGICAL ANALYSIS OF OVARIAN TUMORS AND THE ROLE OF ER, PR AND HER2-neu IN SURFACE EPITHELIAL TUMORS OF OVARY

submitted by Dr. T. PRIYA **of**

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Prof. Dr. AL. SHANTHI, M.D.,
Professor & Head,
Department of Pathology,
Thanjavur Medical College and Hospital,
Thanjavur

Place : Thanjavur

Date : -10-2015

DECLARATION

I, **Dr.T.Priya** solemnly declare that this Dissertation **"CLINICOPATHOLOGICAL ANALYSIS OF OVARIAN TUMOURS AND THE ROLE OF ER, PR, HER-2/neu IN SURFACE EPITHELIAL TUMOURS OF OVARY"** is a bonafide record of work done by me in the Department of Pathology, Thanjavur Medical College and Hospital, Thanjavur under the Guidance and Supervision of Professor **Dr.AL.SANTHI, M.D.,D.G.O**, Head of the Department, Department of Pathology, Thanjavur Medical College, Thanjavur between May 2013 and April 2016.

This Dissertation is submitted to The Tamilnadu Dr. M.G.R Medical University, Chennai in partial fulfilment of University regulations for the award of M.D Degree (Branch – III) in Pathology to be held in April 2016.

Dr.T. Priya,
Postgraduate Student,
Thanjavur Medical College.
Thanjavur.

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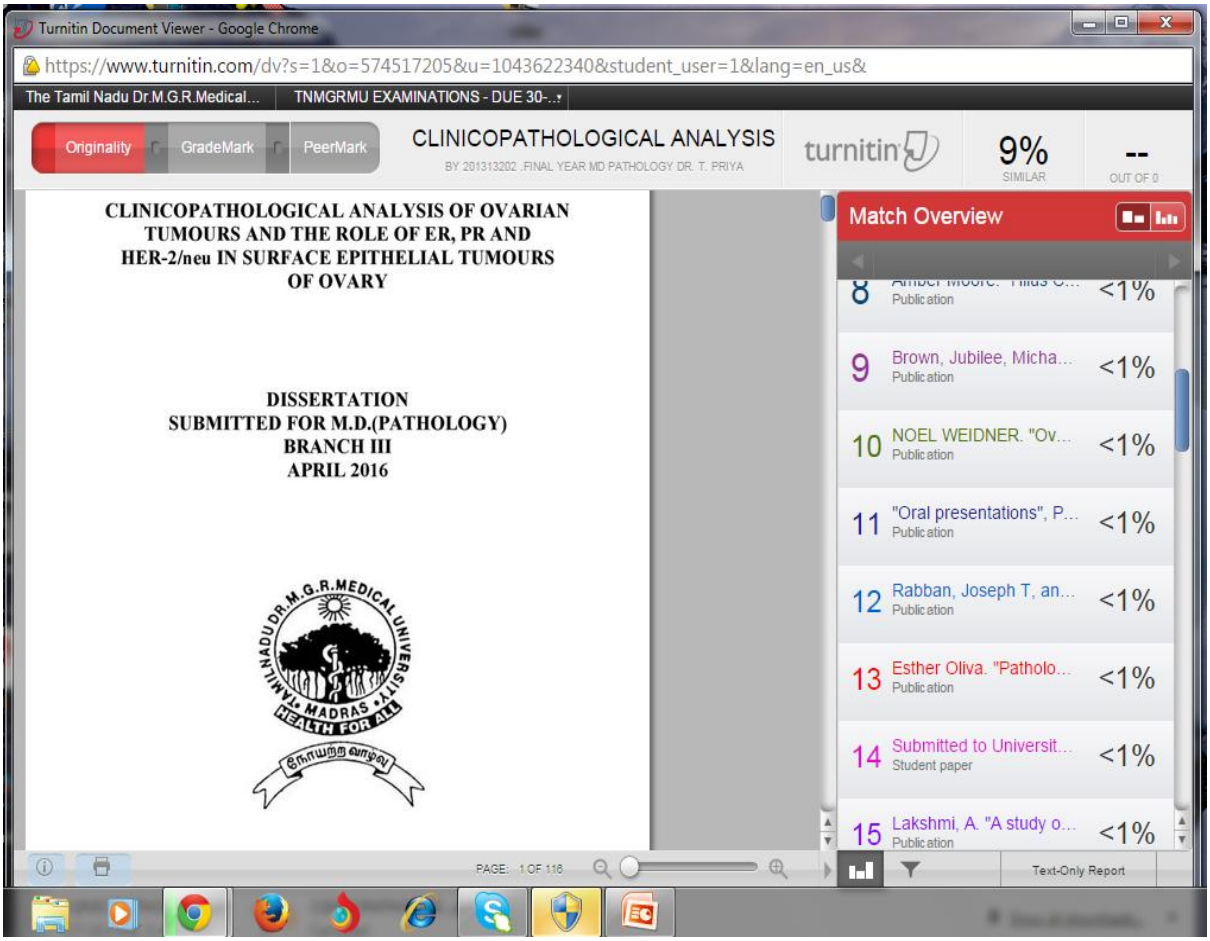
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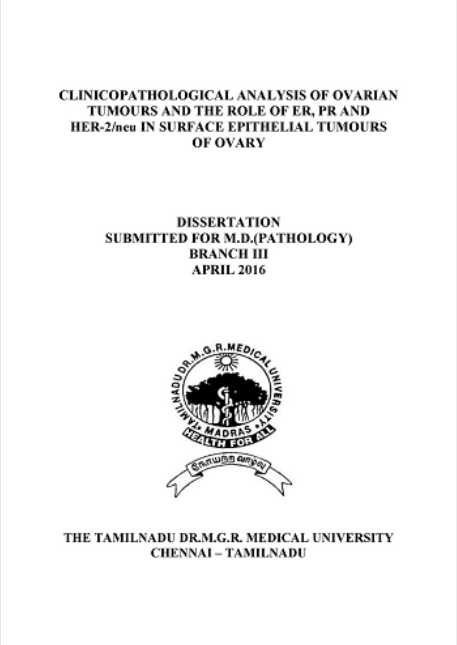

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CONTENTS

ABSTRACT

Ovarian cancer is 6th most common cancer in women worldwide. It has been suggested that incessant ovulation and gonadotrophin stimulation may play a role in development of ovarian cancer. Ovarian cancer carries highest mortality among all gynaecological cancers because the early stages are asymptomatic. The tumour is diagnosed only in late stages (Stage III and IV). No effective screening methods are available as in cervical cancer. Epidemiological evidence suggests that steroid hormones (estrogen and progesterone) and amplification of Human epidermal growth factior-2 (HER-2/neu) gene are implicated in ovarian carcinogenesis. Thus the steroid hormone receptor positivity on the ovarian surface epithelium and ovarian carcinoma is of paramount significance for hormonal therapy. This study is undertaken to review the incidence of ovarian neoplasm in our institution.

A total of 132 cases were evaluated in concordance with clinical history, histopathological features. Immunohistochemistry (ER, PR) was done for surface epithelial carcinomas. HER-2/neu expression was compared between malignant surface epithelial carcinomas and borderline tumours.

Ovarian malignancy (4.1%) was found to be the second most common malignancy in female genital tract next to cervical malignancy. In our institution surface epithelial tumour (68.9%) was the most common neoplasm. Mucinous cystadenoma was the commonest tumour.

Positive ER(36.3%) and PR(45.4%) expression in surface epithelial malignancies proves the mitogenic role of estrogen and progesterone. HER-2/neu showed positive expression in surface epithelial malignancies only (60% serous and 33.3% of mucinous carcinomas). Negative expression was seen in

borderline tumours. Hence HER-2/neu helps in differentiating borderline and malignant tumours.

Thus a panel of markers will be helpful in prognostication of ovarian tumours and development of targeted therapy.

KEY WORDS:

- Ovarian cancer
- Surface epithelial tumours
- ER, PR
- HER-2/neu

ABBREVIATIONS

ER	-	Estrogen receptor
PR	-	Progesterone receptor
HER-2/neu	-	Human epidermal growth factor

INTRODUCTION

Ovarian cancer is the 6th most common cancer in women worldwide. It is the 5th leading cause of death among women in the developed countries[1,2]. The incidence rate is higher in white women followed by Hispanic, Asians, Black and American Indian women[3].

In India, the Age Standardized incidence Rate (ASR) for ovarian Carcinoma varied from 0.9 to 8.4 per 1,00,000 person/year. Studies revealed that the peak incidence is in between the age of 55-64 years. The mean annual percentage increase in ASR ranges from 0.7 to 2.4%[4].

The risk factors associated with ovarian tumours are[5]

1) Age
2) Positive family history
3) Genetic factors
4) Hormonal influences
5) Reproductive factors.

It has been suggested that incessant ovulation and gonadotrophin stimulation may play a role in development of ovarian cancer[6]. Predominantly ovarian neoplasms are sporadic in nature. About 5-10% of ovarian cancers are hereditary. These women have inherited mutations in BRCA-1 and BRCA-2, tumour suppressor genes[5,7].

For diagnosis of early ovarian cancer, abdominal USG and serum CA-125 were used as screening methods. FNAC can be used to differentiate benign from malignant ovarian tumour with an accuracy of 90-95%[8]. Despite these screening methods, ovarian neoplasm carries the highest mortality among all the gynaecological cancers. This is due to the fact that, the early stages are asymptomatic. The tumour is diagnosed only in late stages (stage III & IV). No effective screening methods are available as in cervical cancer.

5 year survival rate is around 40%. In late stages it is only 10-20%. The above scenario is due to paucity of knowledge about exact etiological factors[9]. Histological grading is an important prognostic factor in the surface epithelial stromal tumours. Also sub-typing

of surface epithelial stromal tumours into Benign, Borderline and Malignant has important influence on therapeutic and prognostic point of view.

Following introduction of CA125 in 1981 as biomarker, for epithelial ovarian carcinoma, numerous biomarkers have been emerging substantially[10]. Epidemiological evidence suggests that steroid hormones (estrogen and progesterone) and amplification of human epidermal growth factor-2 (Her-2/neu) gene are implicated in ovarian carcinogenesis[1,2]. Limited number of clinical trails have demonstrated efficacies of anti estrogen and progesterone alone or in combination with chemotherapeutic drugs in the treatment course[1].

Thus the steroid hormone receptor positivity on the ovarian surface epithelium and ovarian carcinoma is of paramount significance for hormonal therapy. The above strategy paves way for novel therapies in the prevention and treatment of ovarian carcinoma[11].

This study is undertaken to review the incidence of ovarian neoplasms in our institution. This study is done with reference to age, histopathological, clinicopathological, immunohistochemical features in concordance with review of journals and various research publications.

AIM OF STUDY

1) To study the incidence of ovarian neoplasms in our institution along with clinical correlation.
2) To study and compare the incidence of malignant ovarian tumour with other female genital tract malignancies in our institution.
3) To study the expression of ER, PR, Her-2/neu amplification status in primary surface epithelial tumour malignancies.
4) To evaluate Her-2/neu expression in distinguishing borderline and malignant surface epithelial tumours of ovary.

MATERIALS AND METHODS

This study is a retrospective study carried out in Department of Pathology, Thanjavur Medical College from January 2013 to May 2015. A total of 132 cases of ovarian neoplasms referred from Raja Mirasudhar Government Hospital (RMH) were included in this study.

The gross specimens were fixed in 10% neutral buffered formalin and processed routinely. Clinical history of the patient including the age, examination findings, radiological investigation, USG, CT and FNAC reports were evaluated in detail.

4 to 5 bits including the wall with papillary excrescences were taken for cystic ovarian neoplasms. 3 to 4 bits were taken in solid tumours of size less than 5cms. In variegated tumours more than 5cms, one block per 1 cm of the tumour was taken in its greatest dimension. Section of 3 to 4μm were cut and stained with hematoxylin and Eosin (Appendix - I).

The H&E stained slides were reviewed. Parameters consisting of age, tumour size, stage of the disease (FIGO Staging) and histological typing were done according to WHO classification criteria. Serous carcinoma were graded according to recent two tier grading system.

The immunohistochemical detection of steroid hormonal status (ER, PR) in surface epithelial stromal tumour were conducted. Also the status of Her-2/neu amplification in borderline and malignant tumours were studied.

ER, PR, Her-2/neu STAINING:

Representative 4μm sections were taken from paraffin embedded blocks for Immuno histochemistry (IHC). The procedure was performed according to heat induced epitope retrieval method with specific antibodies against ER, PR and Her-2/neu.

Scoring for ER and PR expression was based on the following criteria. Proportion of cells exhibiting distinct nuclear immunostaining and intensity of staining was taken into consideration.

The results were given as Negative (<10%) and Positive (≥10%)[12].

Her-2/neu positivity was assessed using Ellis and Wolf recommendations[13].

SCORE	**STAINING PATTERN**
1+	barley perceptible membrane staining in >10% of cells.
2+	weak to moderate complete membrane, staining present in >10% of tumour cells.
3+	strong, complete membrane staining in >10% tumour cells.

RESULTS:

2+	Equivocal
3+	Positive, cytoplasmic staining was considered non-specific.

REVIEW OF LITERATURE

EMBRYOLOGY

Around sixth week of intrauterine life, sexual differentiation can be recognized. The premature gonad is identified as ovary by absence of testicular differentiation. The above process is a passive event. Familial XX gonadal dysgenesis is transmitted as autosomal recessive trait. The above fact suggests, autosomal genes are essential for human ovarian organogenesis. Around 5^{th} week of gestation, gonadal ridges are formed. There after epithelial proliferation and mesenchymal condensation occurs.

Primordial germ cells are the first to develop in the wall of yolk sac. These epithelial cells proliferate to form primitive sex cords. Then they migrate to gonadal ridges. In the surface epithelium there occurs proliferation of second generation of gonadal cortical cords. These traverse the mesenchyme. These cords split to enclose a single primitive germ cell to form follicular cells. The germ cells form oogonia. The coelomic epithelium encloses the developing ovary[14].

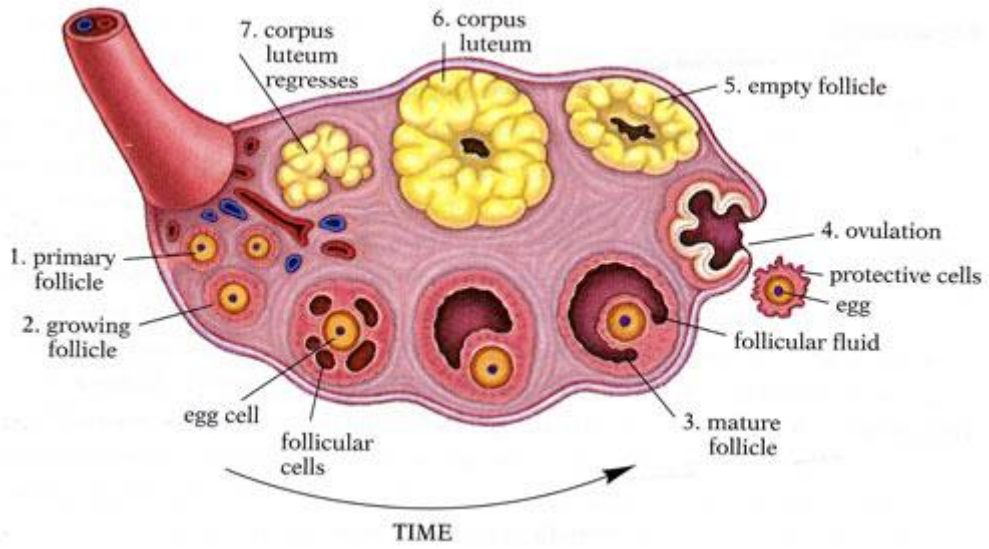
GROSS ANATOMY

Ovaries are paired, almond shaped pelvic organ. They are located one on each side of the uterus, close to the pelvic wall. The ovaries are attached to the posterior aspect of broad ligament by mesovarium(Fig-1). Each ovary measures 4 to 5cm in length, 1.5 to 2.5cm width and 0.5 to 1.5cm thickness. The mature ovary weighs about 5 to 10gms. The postmenopausal atrophied ovary weighs about 3 to 6gms.

Fig.1. Schematic diagram of female genital tract

CUT SURFACE

Reveals a thin white, outer cortex and major areas occupied by medulla. During reproductive period, fluid filled cystic follicles and bright yellow corpora lutea can be seen[15].



VASCULAR SUPPLY:

Major blood supply of ovary is from Ovarian arteries. Ovarian artery is a branch of abdominal aorta. It anastomosis with the ovarian branches of internal iliac arteries in the mesovarium.

VENEOUS DRAINAGE:

The right ovarian vein drains into the inferior vena cava. The left ovarian vein drains into the left renal vein.

LYMPHATICS:

The lymphatics drain into external iliac and paraaortic nodes[7].

HISTOLOGY:

The ovary is covered by coelomic epithelium which is continuous with the mesothelium of peritoneal cavity. The coelomic epithelium is usually a single layer of cuboidal cells, columnar or focally pseudostratified.

Underneath the surface epithelium, outer collagenous zone termed Tunica Albugenia and inner cellular cortex containing follicles are seen. The medulla contains stroma and hilum.

In the cortex lies the primordial follicle which undergoes maturation into primary, secondary, tertiary and Graffian follicles, which releases oocyte during ovulation. Fig.2, 3 The stroma consist of spindle cells exhibiting storiform pattern[7]. The blood vessels gain entrance through hilum. Hilus cells contain crystals of Reinke.

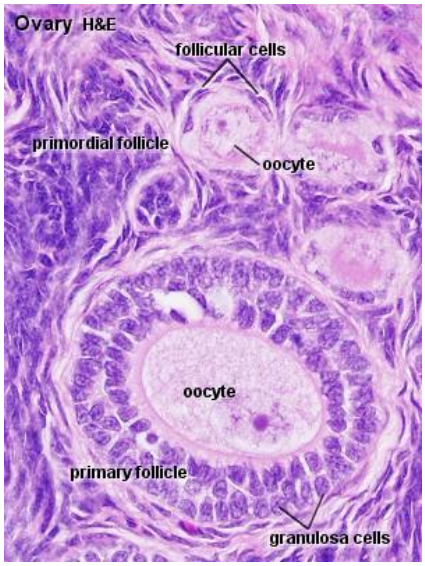


Fig.2. Histology of ovarian cortex

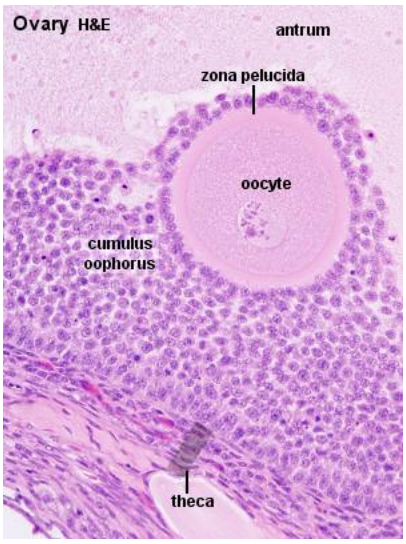


Fig.3. Histology of Graffian follicle

FUNCTIONS

Ovaries produce ova and hormones under the feedback control mechanisms of hypothalamo-pituitary ovarian axis through endocrine, paracrine and autocrine pathways.

RISK FACTORS FOR OVARIAN CARCINOMA

Neoplasms of ovary constitutes about 30% of all cancers of female genital tract. The age adjusted incidence rate is highest in well developed countries.

The factors that risk ovarian carcinoma are three fold.

1. Environmental
2. Genetic
3. Life style.

Higher socio-economic status is included as one of the risk factors.

About 5 - 10% of cases are familial. There is three times increased risk in individuals with positive family history. Majority are due to mutation in BRCA1, BRCA2 and DNA mismatch repair genes. Other syndromes which are associated with ovarian cancer are Lynch-II syndrome and Hereditary Non Polyposis Colon Cancer Syndrome(HNPCC)[16].

Nulliparity, early menstruation, late menopause and ovarian inflammation are more commonly associated with increased risk for ovarian tumours. The above repeated traumatic insults might be the cause of ovarian cancers. Oral contraceptive use, multiparous women, pregnancy, lactation reduces the risk by reducing the ovulation process.

Clomiphene citrate, long term estrogen replacement therapy, obesity favour ovarian carcinogenesis. Diet rich in cheese, meat increases the risk. Beverages like tea and vegetables like tomato consumption reduces the risk[17].

CLINICAL FEATURES

- Age > 40 Years
- Abdominal mass
- Abdominal pain
- Ascites
- In functional ovarian tumours, precocious puberty, menstrual irregularities[17].

In high risk individuals serum biomarker assay, transvaginal ultrasound and genetic studies can be used as screening test[18].

SPREAD AND METASTASES

Modes of spread

- Direct
- Hematogenous
- Lymphatic route.

The metastatic sites are

- Contralateral ovary
- Peritoneal cavity
- Paraaortic lymphnodes
- Liver
- Lung [Yolksac & Choriocarcinoma] by hematogenous spread[16].

CLASSIFICATION

Novak, in 1967 classified ovarian tumours as benign/malignant and cystic/solid. The advantage being simple. Disadvantage is that the borderline tumours fell into grey zone[19]. WHO (World Health Organization) in the year 1973 formulated classification based on histogenesis. The above classification was updated in 1999 and 2003[7,20].

WHO CLASSIFICATION OF TUMOURS OF THE OVARY.

I. SURFACE EPITHELIAL – STROMAL.

Serous tumours

- Malignant
- Adenocarcinoma

- Borderline tumour
- Benign – cystadenoma, adenofibroma, cystadenofibroma.

Mucinous tumours:

- Malignant
- Adenocarcinoma
- Borderline tumour
- Benign – Cystadenoma, adenofibroma, cystadenofibroma
    - Mucinous cystic tumours with pseudomyxoma peritonei

Endometrioid tumours:

- Malignant
- Adenocarcinoma
- Malignant mixed mullerian tumour
- Endometrial stromal sarcoma.
- Benign – cystadenoma, adenofibroma, cystadenofibroma.

Clear cell tumours:

- Malignant
- Adenocarcinoma
- Borderline tumours
- Benign – Cystadenoma, adenofibroma, cystadenofibroma.

Transitional tumours

- Malignant
- Transitional cell carcinoma (non-Brenner type)
- Malignant Brenner tumour
- Borderline
- Benign Brenner tumour

Squamous cell carcinoma

Mixed epithelial tumours

- Malignant
- Borderline
- Benign

Undifferentiated and unclassified tumours:

- Undifferentiated carcinoma
- Adenocarcinoma, not otherwise specified.

II. SEX CORD – STROMAL TUMOURS.

Granulosa-stromal cell tumour

- **Granulosa cell tumour**
    - Adult Granulosa cell tumour
    - Juvenile granulosa cell tumour
- **Thecoma Fibroma group**
    - Thecoma, not otherwise specified.
    - Typical
    - Luteinized.
- **Fibroma**
    - Cellular Fibroma
- **Fibrosarcoma**
- **Stromal tumour with minor sex cord elements**
- **Sclerosing stromal tumour**
- **Signet-ring stromal tumour**

Sertoli-Stromal cell tumours:

Sertoli Leydig cell tumour group.

- Well differentiated
- Of intermediate differentiation variant with heterologous elements
- Poorly differentiated variant with heterologous element.
- Retiform variant with heterologous elements.
- Sertoli cell tumour
- Stromal –Leydig cell tumour

SEX CORD – STROMAL TUMOURS OF MIXED OR UNCLASSIFIED CELL TYPE.

- Sex cord tumour with annular tubules
- Gynandroblastoma
- Sex cord stromal tumours, unclassified.

Steriod cell tumours

- Stromal luteoma
- Leydig cell tumour group

  Hilus cell tumour

  Leydig cell tumour, non hilar type

  Leydig cell tumour, not otherwise specified.
- Steroid cell tumour, not otherwise specified

  Well differentiated

  Malignant

III. GERM CELL TUMOURS

- Primitive Germ cell tumours
- Dysgerminoma
- Yolk Sac tumour

- Embryonal carcinoma
- Polyembryoma
- Non Gestational Choriocarcinoma
- Mixed Germ cell tumour
- Biphasic or Triphasic Teratoma
- Immature Teratoma
- Mature Teratoma
    - Solid
    - Cystic
    - Fetiform Teratoma.
- Monodermal teratoma and somatic type tumours associated with dermoid cyst
- Thyroid tumour groups
    - Struma Ovarii
- Benign
- Malignant
- Carcinoid tumour
- Neuroectodermal tumour group
- Carcinoma group
- Melanocytic group
- Malignant melanoma
- Melanocytic nevus
- Sarcoma group
- Sebaceous tumour group
- Pituitary type tumour group
- Retinal anlage tumour group.

Germ Cell Sex Cord – Stromal Tumour

- Gonadoblastoma – Variant with malignant germ cell tumour.
- Mixed germ cell – sex cord – Stromal tumour variant with malignant germ cell tumour.

IV. TUMOURS OF THE RETE OVARII:

- Adeno carcinoma
- Adenoma
- Cystadenoma
- Cystadeno fibroma

V. MISCELLANEOUS TUMOURS:

- Small cell carcinoma hypercalcemic type
- Small cell carcinoma, pulmonary type
- Large cell neuroendocrine carcinoma
- Hepatoid carcinoma
- Primary ovarian mesothelioma
- Wilms tumour
- Gestational choriocarcinoma
- Hydatidiform mole
- Adenoid cystic carcinoma
- Basal cell tumour
- Ovarian Wolffian tumour
- Paraganglioma
- Myxoma
- Soft tissue tumours, not specific to the ovary.

VI. TUMOUR LIKE CONDITIONS

- Luteoma of pregnancy
- Stromal hyperthecosis
- Stromal hyperplasia.
- Fibromatosis
- Massive ovarian edema

VII. LYMPHOID AND HAEMATOPOIETIC TUMOURS

VIII. SECONDARY TUMOURS

I. SURFACE EPITHELIAL – STROMAL TUMOURS:

The cell of origin is from surface epithelium, or serosa of ovary. 60% of ovarian neoplasm, and 80-90% of primary ovarian malignancies belong to this group. The benign epithelial tumours are common in middle age. The malignant epithelial tumours are common in perimenopausal age group[16].

MOLECULAR PATHOGENESIS OF SURFACE EPITHELIAL TUMOURS

High grade serous and Endometrioid carcinomas arise from invagination of surface epithelium (inclusion cyst). This is associated with P53, BRCA1 and/or BRCA2 mutations. Low grade serous carcinoma arise from the mutation of RAS-RAF pathway. It is also associated with adenoma – borderline neoplasm-carcinoma sequence. Mucinous carcinomas arises in the background of mutation in K-ras oncogene. Low grade Endometrioid carcinoma arise in the background of endometriosis and mutations in CTNNBI (gene encoding Beta Catenin) and PTEN[21]. Based on cell type epithelial tumours are classified as

- Serous
- Mucinous
- Endometriod
- Clear Cell

Based on pattern of growth

- Cystic
- Solid

Based on atypia/invasiveness

- Benign
- Borderline
- Malignant tumours

A) SEROUS TUMOURS:

Serous tumours constitute the most common surface epithelial neoplasms. As a result of mullerian differentiation along the salphingeal pathway, the cells resemble that of tubal epithelium[7].

Serous tumours constitute 30% of all ovarian tumours out of which, 70% are benign, 5-10% borderline and 20-25% are malignant[16,22].

BENIGN SEROUS TUMOURS:

Incidence is more common in fifth decade.

Gross:

Bilaterality is seen in 10-20%. Usually unilocular, filled with clear serous fluid. They may be cystadenomas / adenofibroma / cystadenofibromas. Cystadenomas are cystic neoplasm with smooth inner surface, sometimes with papillary excrescences, hence called papillary serous cystadenoma. Adenofibroma contain a dense fibrous stromal component. Cystadenofibroma contain polypoidal excrescences embedded in a fibrous stroma.

Microscopy:

Cyst and polypoidal excrescences are lined by single layer of ciliated epithelium similar to fallopian tube. No nuclear atypia is seen[16,22].

SEROUS BORDERLINE TUMOUR:

Synonyms: Atypical proliferating tumour or serous tumours of low malignant potential.

Gross:

These tumours are bilateral in 26-36% of cases. Grossly they usually present as cystic. The inner surface showing more friable and numerous papillary projections. Cystadenofibroma has white to yellow rubbery areas.

Microscopy:

Diagnostic features:

(1) Atleast 10% of the areas exhibit arborising papillae (hierarchical branching) with epithelial stratification.

(2) Varying degrees of mild to moderate nuclear atypia.

(3) Absence of destructive stromal invasion.

Serous borderline tumours are classified as

(1) Typical (90%)

(2) Micropapillary (10%)

The typical type has classical branching papillary structures with epithelial tufts overlying the papillae.

The micropapillary type has thin elongated papillae. These papillae are five times longer than they are wide. They have no stromal support. They arise directly from the papillae with thick fibrous stalk.

The following features should be looked for, after the diagnosis

(1) Surface involvement

There is higher risk of transformation to higher stage in this category.

This also enables peritoneal spread.

(2) Stromal microinvasion

Microinvasive type has micropapillary structures and discohesive epithelial cells. They are surrounded by clear space, in the underlying normal tissue. This invasive foci which is measuring less than $10mm^2$ and less than $3mm^2$ in greatest dimension is called as microinvasion.

(3) Lymph node metastases

Metastases are seen in pelvic and paraaortic lymphnodes in 27% of cases.

(4)Peritoneal implants**:**

In 30% of cases, serous borderline tumours are associated with implants in the peritoneum. Classified as invasive and noninvasive types.

Invasive implants:

(a) Haphazardly arranged glands are seen in normal tissues like omentum.

(b) Dense fibrous reaction without inflammation are seen surrounding the implants.

(c) Epithelial proliferation is seen.

(d) Nuclear features are similar to low grade serous carcinoma.

(e) Borders are irregular.

(f) Aneuploidy.

These above features are absent in noninvasive implants.[57]

Even in the presence of invasion the prognosis seems to be good. Because they behave as noninvasive neoplasms. In child bearing group, conservative surgery is performed along with close clinical followup.[23,24,25,57]

MALIGNANT SEROUS CYSTADENOCARCINOMA

It is the most common malignant ovarian neoplasm. It constitutes about 40-50% of total.

Gross:

These tumours are usually large, bilateral (70%). Size ranging from microscopic foci to more than 20cms in diameter. They present as cystic with papillary excrescences, solid growth and growth on the surface due to capsular invasion. Large areas of hemorrhage and necrosis are also seen.

Well differentiated tumours have well formed papillary structures. They have characteristic fibrovascular core. Psammoma bodies are common in the fibrovascular stalk. When 75% of the tumour shows psammoma bodies they are called psammoma carcinoma. They carry favourable prognosis. Moderately differentiated tumour shows crowded papillae without fibrovascular core. Poorly differentiated types shows no papillary pattern. The cells are arranged in solid sheets.

The papillae has to differentiated from papillae occurring in Transitional cell carcinoma, Endometrioid carcinoma, clear cell carcinoma. In Endometrioid carcinoma, there is villous structure with focal squamous metaplasia. In clear cell carcinoma, the papillae are lined by hob nail/clear cells. The papillae have hyalinised core. In transitional carcinoma, the papillae are broad and lined by transitional cells[7,6,55].

Immunostaining shows CK7 positivity, CK20 negativity, WT1, P53 expression[31]. Hormonal markers ERα, PRα showed higher expression in malignant cases. Her-2/neu was found to show higher expression for malignant tumours when compared to borderline[26].

GRADING:

The most commonly used Silverberg's grading system[27]:

Table:1:

Score	Architecture	Cytological atypia	Mitotic figures/10Hpf.
1	Glandular	Mild	0-9
2	Papillary	Moderate	10-24
3	Solid	Severe	>25

Score 3-5 - Grade 1

Score 6-7 - Grade 2

Score 8-9 - Grade 3

2-Tier Grading system for serous carcinoma

Anais Malpica *et al*. classifies serous tumour into low and high grade based on nuclear atypia and mitotic rate[52].

Low grade:

1. Mild to moderate nuclear atypia and as a secondary feature.
2. <12 mitosis/10Hpf.

High grade:

1. Marked nuclear atypia and as secondary feature.
2. >12 mitosis/10Hpf and multinucleated cells.

B) MUCINOUS TUMOURS:

Mucinous tumours constitute 14% of all ovarian tumours. Mucinous tumours are the second most common ovarian tumours. About 30.8% of all surface epithelial tumours belong to this category. Of these, 75% are benign, 20% are borderline, <5% are invasive carcinomas. 5% are bilateral.[16,28]

BENIGN MUCINOUS TUMOURS

Gross:

Usually they are large, unilateral, multilocular. The multiloculated cyst contains viscous mucoid material. Cystadenofibromas present as solid tumours.

Microscopy:

Depending on the lining epithelium of cyst they are classified as:

1) Intestinal

2) Endocervical type.

Endocervical type resembles endocervical epithelium. They have ciliated columnar epithelium with intracellular mucin. Intestinal type epithelium consists of goblet cells, paneth cells exhibiting picket fence appearance. Cystadenofibromas present as mucinous glands / cyst distributed in dense fibrous stroma.[29]

BORDERLINE MUCINOUS TUMOURS

The lining epithelium is stratified not more than 3 layers. They form filiform intracystic papillae with minimal stromal support. Nuclear atypia and mitotic figures are noted. Stromal microinvasion is a feature. This consists of isolated cells/clusters. Each cluster is composed of 5-10 cells without stromal reaction[28,30].

MALIGNANT MUCINOUS OVARIAN TUMOUR

Synonym : Mucinous cystadenocarcinoma

Gross:

They are mostly unilateral. About 5% of these tumours are bilateral. If bilateral, nonovarian origin should be excluded. These tumours are common in 40 to 80 years of age. They are large and have smooth external surface. Cut surface reveals many-thick walled multilocular cystic spaces. The cyst contains viscous fluid. Solid areas, hemorrhagic areas and necrosis are not infrequent.

Microscopy:

The lining epithelium is stratified with more than 4 layers. Two forms of stromal invasion noted as:

(1) Expansile

(2) Infiltrative.

In expansile form, back to back arrangement of glands are seen with no stroma in between. The infiltrative type shows glands, tubules, cords, cell nest haphazardly infiltrating the stroma[7,16].

Differential Diagnosis:

Metastatic colon adenocarcinoma:

They are mostly bilateral. Nodular growth pattern, surface involvement and lymphovascular invasion are commonly associated. They are CK20 positive and CK7 negative[31].

MUCINOUS TUMOUR WITH PSEUDOMYXOMA PERITONEI

It includes mucinous ovarian neoplasm, ascites, abundant gelatinous material within pelvis and abdominal cavity. We must always rule out the possibility of appendiceal neoplasm or other gastrointestinal primary mucinous tumours metastatic to peritoneum[31].

MIXED OR COLLISION TUMOUR

This is due to biphasic or multiphasic differentiation. Two or more epithelial elements are present in varying proportions. The above entity is also seen in sex cord stromal and germ cell tumours. Commonly benign mucinous tumours occur in association with the mature cystic teratoma[7,16].

c) ENDOMETRIOID TUMOURS:

BENIGN ENDOMETRIOID TUMOURS

Gross:

They are solid, firm, tan with numerous cyst of varying sizes. The tumours are ranging from 8 to 10cms in diameter.

Microscopy:

Benign appearing glands/cyst are seen. These structures are lined by endometrial type of cells[32]. They are sometimes associated with squamous differentiation.

BORDERLINE ENDOMETRIOID TUMOURS:

Gross:

Mostly unilateral ranging from 2-40cms in diameter. Cut surface is grey white, tan. Predominantly these tumours are solid. Large tumours, may show areas of hemorrhage and necrosis .

Microscopy:

Crowded Endometrioid glands/cyst are seen. There is cytological atypia and low mitotic activity is seen. But characteristically, there is no invasion into the stroma. 15-50% of patients have incidence of same side ovarian endometriosis or at other extra ovarian sites[32,60].

ENDOMETRIOID ADENOCARCINOMA

Hyperestrogenic state, atypical hyperplasia within a foci of endometriosis serve as risk factor for Endometrioid carcinoma. This suggests the origin of tumour is directly from ovarian coelomic epithelium. This entity has a very good prognosis[33].

Gross:

They present as solid, cystic friable papillae or as a mural nodule. These tumours range from 10 - 20cms diameter. Bilaterality is seen in 28% of cases.

Microscopy:

TABLE 2:

FIGO grading scheme for Endometrioid adeno carcinoma:

Grading	Histological feature
Grade 1 or well differentiated	Well formed glands resembling villoglandular carcinoma of uterine corpus,<5% of solid tumour growth.
Grade 2 or moderately differentiated	More complex, glandular architecture, increased nuclear stratification, 6-50% solid tumour growth.
Grade 3 or poorly differentiated	Poorly formed glands, large sheets of cells, >50% solid tumour growth.

Squamous differentiation is seen in 30% of cases. The differential diagnosis is metastatic uterine endometrial carcinoma. Endometrioid carcinoma of ovarian origin may have associated endometriotic foci. The secretory change, squamous metaplasia, expansile invasion are evident .These tumours shows beta catenin, EMA positivity[31].

·CLEAR CELL (MESONEPHROID) TUMOURS

Clear cell carcinomas are more common in nulliparous women[7]. They constitute 5% of all ovarian tumours. Clear cell carcinomas are most commonly associated with pelvic and endometrial endometriosis[33].

Clear cell tumours are classified into benign, borderline, malignant.

Gross:

Thick walled unilocular cyst is seen. Numerous fleshy yellow nodules project into the cyst are noted.

Microscopy:

They may present as solid mass, cystic or papillary. The cores of papillae exhibit prominent hyalinisation. The papillae are lined by one or two layers of polygonal/flattened/or characteristic hobnail cells. The individual cells have clear cytoplasm containing glycogen. The nuclear atypia is minimal[34].

Differential diagnosis include

(1) Dysgerminoma which has polygonal shaped cells, inconspicuous nucleoli, presence of luminal mucin and EMA positivity.

(2) Metastatic renal cell carcinoma should also be considered. The clear cell renal cell carcinoma is CD10 positive, CK7 negative and vice-versa in clear cell carcinoma of ovary.

D) BRENNER TUMOUR (TRANSITIONAL CELL TUMOUR):

Brenner tumour accounts for 1-2% of all ovarian neoplasm. The average age of presentation is about 50 years. Bilaterality is seen in 5 to 7% of the cases[29].

The cell of origin is from surface epithelial cell or cyst derived from them. This is through the process of metaplasia. These tumours are rarely associated with mucinous cystadenoma and with Transitional cell tumour of bladder.

BENIGN BRENNER TUMOUR

Gross:

Predominantly these tumour are well circumscribed. Cut surface-firm, white or yellowish measuring about 2 to 30cms diameter. Focal areas of calcification may be seen. 6% of the cases are bilateral.

Microscopy:

Nests of transitional epithelial cells resembling the urothelium is seen. This is surrounded by abundant dense fibroblastic stroma. Individual cells have oval nuclei with longitudinal groove resembling coffee bean. The cells have clear cytoplasm. Cystic change can be seen[34].

BORDERLINE BRENNER TUMOUR:

Gross:

Large, with an average diameter 16-20cms. Cut surface shows solid and cystic components.

Microscopy:

Branching papillae lined by transitional epithelium protruding into cystic space. Spindle to oval cells without mitotic activity is seen. There is no stromal invasion. A benign Brenner component is always present[34].

MALIGNANT BRENNER TUMOUR

Gross:

They are large tumours. Cut surface reveals predominantly solid and few cystic areas.

Microscopy:

These neoplasm exhibits stromal invasion. This is accompanied by a benign or borderline Brenner component. The invasive element present as high grade transitional cell carcinoma[35].

TRANSITIONAL CELL CARCINOMA:

They resemble transitional cell carcinoma of urinary tract in architecture. Most commonly seen in age group of 50 to 58 years.

Gross:

Bilateral in 15% of cases. Cut surface reveals partly solid and cystic areas. Occasional polypoidal projections accompanied by areas of hemorrhage and necrosis is seen.

Microscopy

Characteristic papillary structures lined by multilayered transitional epithelium is seen. The cells exhibit pleomorphism. These tumour should not have a benign/borderline Brenner component[35].

This type of neoplasm has got a good prognosis. The differential diagnosis of metastatic tumours from urinary tract should always be considered. Transitional tumours of urinary tract show classically CK 20 positive, which is absent in tumours of ovarian origin[31].

E) MALIGNANT MIXED MULLERIAN TUMOURS

Gross:

90% of these tumours are bilateral. External surface - Bosselated mass, large tumors. Cut surface - Partly solid and cystic. Areas of hemorrhage and necrosis are seen.

Microscopy:

It is a biphasic neoplasm. It is composed of both malignant epithelial and mesenchymal component. Any of the surface epithelial tumour constitute the epithelial component. The mesenchymal component is fibrosarcoma / leiomyosarcoma / Endometrioid stromal sarcoma[34].

F) ADENOSARCOMA

Gross:

Always unilateral. Cut surface – predominantly solid. Small cysts are occasionally seen.

Microscopy:

Biphasic tumour in which the epithelial component is benign. But the mesenchymal component is malignant. The cellular stroma shows periglandular cuffing pattern. The adenosarcoma of ovary has grave prognosis in comparison with its uterine counterpart[31].

G) ENDOMETRIAL STROMAL SARCOMA:

Gross:

About 70% of tumours present as unilateral tumours. Predominantly the cut surface is solid. Cystic areas filled with mucoid and hemorrhagic fluid material may be seen.

Microscopy:

50% of cases occur in association with endometriosis. The characterstic features of numerous thick walled blood vessels are seen. The tumour cells are arranged in whorls around blood vessels or in diffuse pattern. The individual cells have round to oval nuclei with scanty cytoplasm[31].

H) MIXED EPITHELIAL TUMOUR

Microscopy:

Varying proportions of two or more of five major cell types are seen: serous, endometrioid, mucinous, clear cell, and transitional types are seen[34].

I) UNDIFFERENTIATED CARCINOMA

Microscopy:

They are uncommon ovarian neoplasm. They have no differentiation. Marked cytolological atypia is seen.

II. SEX CORD STROMAL TUMOURS:

5% of ovarian neoplasms belong to this group of functional tumours. The cell of origin is from the sex cord, parenchyma or both of the embryonic gonads. The neoplastic cells differentiate into either testicular (sertoli-leydig cell tumours) or ovarian type (Granulosa cell tumours)[16,53]. The sensitive and specific immuno marker for sex cord stromal tumours are inhibin and calretinin [31].

A) GRANULOSA STROMAL CELL TUMOUR:

They constitute 1 – 2% of ovarian tumours. 70% of sex cord stromal tumours belong to this group of potentially malignant tumours. The cell of origin is follicular granulosa cells. Two types:

1) Adult Granulosa cell tumour
2) Juvenile Granulosa cell tumour

ADULT GRANULOSA CELL TUMOUR:

More common in the reproductive age group. 40% of the cases are seen in the menopausal age group. About 75% of the patients present with abnormal uterine bleeding due to hyperstrogenism.

Gross:

These tumours have an average diameter of 12cms. Cut surface - Yellow to greyish white in colour, solid and cystic areas are seen. Cystic area filled with hemorrhagic fluid is seen. Areas of hemorrhage and necrosis may be seen exhibiting a variegated appearance.

Microscopy:

Growth patterns of microfollicular with characterstic Call Exner bodies, Macro follicular, insular, trabecular, watered silk,diffuse, solid, sarcomatoid are seen. Recent studies suggest the tumour size and mitotic index has more prognostic significance than the pattern[34,36].

The individual granulosa cells have scanty cytoplasm with longitudinal nuclear grooves giving rise to coffee bean appearence. A low mitotic activity and minimal to no cytological atypia is noted.[22] The granulosa cells are often surrounded by fibro thecomatous stroma(fibroblasts, theca/leutinised cells). The morphology and inhibin positivity strongly suggests granulosa stromal cell tumour[31,58].

JUVENILE GRANULOSA CELL TUMOUR:

80% of these tumours are seen in adolescent girls. Often they present with complaints of precocious puberty. The above entity may be seen in association with olliers disease/mafucci's syndrome.

Gross:

Lobulated external surface is seen. Cut surface reveals solid to cystic areas. The solid areas are yellow to tan. They are soft in consistency.

Microscopy:

Macrofollicular pattern are more common. Neoplastic granulosa cells are having abundant eosinophilic vacuolated cytoplasm. There is no grooving. Numerous mitotic figures

are evident. They present with distant metastasis. The patients have grave prognosis when compared to adult granulosa type[37].

B)THECOMA-FIBROMA GROUP:

a) THECOMA:

4% of all ovarian tumours belong to this group. They occur in post menopausal age group[38].

Gross:

They are almost and always unilateral. An average size of 5-10cms in noted. Cut surface - solid, grey tan to yellow areas with well defined capsule. Firm in consistency.

Microscopy:

Fasicles of bland oval to spindle shaped cells are seen. Nuclei are fusiform with abundant pale vacuolated lipid rich cytoplasm. Calcification is prominent. Leutinised thecoma cells are also evident. They contain leutin cells, in a background of fibromatous than thecomatous stroma. Edema and microcyst formation are commonly seen. Mitotic figures are not seen.

Special stains like oil red O demonstrates intracytoplasmic neutral fat. Silver stains like reticulum, stains around each tumour cell in contrast to granulosa cells in which the reticulin staining is around the cluster of cells[35].

b) FIBROMA:

Common ovarian tumour occurring around puberty. The patients are asymptomatic. Fibromas are incidentally discovered during surgery. Ovarian fibroma along with ascites and right sided pleural effusion constitute Meig's syndrome. In Gorlen's syndrome they present bilaterally[39.40].

Gross:

Most commonly they are unilateral. Average size is around 6cms in diameter. Cut surface - solid, white, lobulated appearance. Hard in consistency.

Microscopy:

These tumours are composed of spindle shaped cells in interlacing fascicles. They are also seen in storiform pattern admixed with dense collagen. The individual cells have uniform bland fusiform nuclei with pointed ends and scant cytoplasm. Mitosis is absent. 10% of tumours have uniform densely cellular and are referred to as cellular fibromas[39.40].

C) SCLEROSING STROMAL TUMOUR OF OVARY

These are benign uncommon neoplasm seen in reproductive age group.

Gross:

Unilateral, well circumscribed with an average of 15cms in diameter. Cut surface shows solid, grey white, lobulated with specks of yellow areas.

Microscopy:

Characteristic pseudolobular pattern of growth is seen. It has alternating hyper cellular and hypocellular areas. Hypercellular areas shows numerous thin walled blood vessels, similar to hemangiopericytoma like pattern. It is composed of collagen producing spindle cells and lipid containing round cells. The hypocellular areas is composed of edematous sclerotic fibrous stroma.

Massive Edema of ovary is an important differential diagnosis. Normal ovarian structures are seen admixed with edematous areas in Edema of ovary. Where as in sclerosing stromal tumour, the normal ovarian stroma is replaced by the neoplasm[7,16].

D) SERTOLI STROMAL CELL TUMOUR

Tumour consisting of varying proportion of sertoli cells, Leydig cells and stromal components are seen.

Sertoli Leydig cell group:

These rare tumours constitute less than 0.1% of ovarian tumours[22]. The tumours are more common in younger age group. The patient presents with masculinisation or

defeminisation features. Previously these tumours were referred as Arrhenoblastoma/Androblastoma.

Gross:

Most commonly they are unilateral. Solid, firm with pale yellow areas admixed with few areas of hemorrhage and necrosis are seen.

Microscopy:

1. MEYER'S type I - Well differentiated tumours.

   Sertoli cells are seen in open or closed tubules. They lack significant atypia/mitotic activity.

2. MEYER'S type II – Intermediate

   Composed of cords, sheets and nests of sertoli like cells separated by spindle shaped stromal cells.

3. MEYERS type III - Poorly differentiated/Sarcomatoid

   They are arranged in sarcomatoid pattern with masses of spindle cells[27].

20% of tumours exhibit heterologous elements. They consist of mucinous epithelium of gastrointestinal tract, skeletal muscle, cartilage and neuroendocrine cells.

E) SERTOLI CELL TUMOURS

They are rare neoplasms. Sertoli cell tumours are more common in reproductive age group.

Gross:

Unilateral in presentation. Average size is 5 to 7cms in diameter .

Microscopy:

The neoplasm is composed of sertoli cells lining the tubules or grow in nests or solid sheets. The individual cells are columnar, polygonal, in shape with small round to oval nuclei with minimal nuclear atypia. They have granular to eosinophilic cytoplasm[27].

F) SEX CORD TUMOUR WITH ANNULAR TUBULES

These tumours may occur as solid, bilateral and multifocal tumours accompanied by Puetz Jegher's syndrome. They are present as solitary neoplasm without the syndrome. Half of the patients present with hyperestrogenism.

Microscopy:

Complex annular tubules filled with eosinophilic material are seen. Occasional hyaline bodies and calcification are also seen. In several tumours the above tumour merged with that of granulosa cell tumour[7,16].

G) GYNANDROBLASTOMA

Mixture of granulosa cell tumours (estrogenic) and sertoli–Leydig cell tumours androgenic features are seen.

Gross:

Unilateral with an average size of 1 to 18cms is seen.

Microscopy:

Equal proportion of sertoli leydig cell and granulosa cell tumour are seen. Tubules and trabeculae of well differentiated sertoli-leydig cells are seen admixed with nests and sheets of granulosa cells[16].

H) LIPID CELL (LIPOID, STEROID CELL):

These tumours accounts for 0.5% of all ovarian neoplasm. Lipid cell tumours are seen in all age groups. They occur in association with defeminisation and virilisation syndromes[22].

Gross:

Unilateral, Well circumscribed. Cut surface – yellowish nodules separated by fibrous septa.

Microscopy:

Tumour cells are arranged in solid sheets. The individual tumour cells are large polyhydral with abundant eosinophilic or vacuolated cytoplasm. The cytoplasm stains positivity for fat stains[27]. 25% of these tumours turn into malignancy. They are characterised by necrosis and hemorrhage. Nuclear atypia and mitotic activity is increased in such cases[16].

III. GERM CELL TUMOURS

30% of ovarian neoplasm come under this category. The most common neoplasm to occur in younger age group (< 18 years) are germ cell tumours. Malignant germ cell tumour (3% of germ cell tumours) are more common in much younger age group[27].

Benign cystic teratoma is the commonest type. They constitute 95% of germ cell tumours[41]. The cell of origin is from germ cell which has undergone defective meiotic division[16]. 8% of the cases are composed of two or more subtypes. They are grouped as malignant mixed germ cell tumours.

A) DYSGERMINOMA:

They constitute 1% of all ovarian cancers. Bilaterality is around 10%. It is the most common malignant tumour in association with gonadal dysgenesis. The patients present with complaints of abdominal mass and pain.

Gross:

Well encapsulated tumours. The average size is 15cms diameter. Cut surface reveals solid, lobular configuration, tan or white in colour. Soft in consistency.

Microscopy:

The tumour cells are found in lobules/sheets of cells with thin fibrous septa separating them. The fibrous septa is densely infiltrated by lymphocytes. The individual tumour cells are polygonal cells with clear to eosinophilic cytoplasm (PAS positive). Round vesicular nucleus and central prominent nucleoli is seen. In 5% of cases syncytiotrophoblasts are seen[16,41].

Anaplastic dysgerminoma resembles embryonic carcinoma. The features of pscudoglandular/tubular pattern, high mitotic activity, high nuclear atypia is seen[41].

Immunohistochemistry markers of CD117 and PLAP (Placental Alkaline Phosphatase) are positive[31].

B) YOLK SAC TUMOUR (ENDODERMAL SINUS TUMOUR):

20% of primitive germ cell tumours belong to this category. These tumours are common in younger age group. The patient present with complaints of abdominal pain and mass.

Gross:

They present as unilateral mass. The average diameter is 15cms. Cut section reveals soft, grey yellow areas with large areas of hemorrhage and necrosis. Cystic areas are not infrequent[42].

Microscopy:

Many patterns of reticular, solid, festoon, hepatoid, pseudo papillary, polyvesicular vitelline are seen. The characteristic schiller duval bodies are seen in 10 to 20% of tumours. They are described as papillae lined by tumour cells that project into dilated cystic spaces giving rise to glomeruloid bodies . PAS positive eosinophilic hyaline globules are seen[42].

Clear cell carcinoma should be considered in the differential diagnosis. The nuclei in yolk sac tumour appears primitive. The papillae of yolksac tumour lack hyalinised core[32].

Yolk sac tumours are positive for cytokeratin, Alpha1Antitrypsin[31].

C) EMBRYONAL CARCINOMA:

These tumours constitute 3% of germ cell tumours. They are most common in younger age group. These tumours present as a component of mixed germ cell tumours. The patient present with features of precocious puberty, vaginal bleeding, hirsutism and amenorrhea.

Gross:

External surface is smooth. Cut surface shows, solid, variegated areas of hemorrhage and necrosis[16].

Microscopy:

Large primitive appearing cells in solid sheets and nests and also forming abortive glandular structures are seen. Most commonly syncytiotrophoblastic giant cells are seen[43].

Serum alpha feta protein and chorionic gonadotrophin are elevated giving false positive pregnancy tests[31].

These tumours have resemblance with poorly differentiated adenocarcinoma or undifferentiated carcinoma. The latter are more common in old age/reproductive age group.

D) POLYEMBRYOMA

Embryonal carcinoma with numerous embryonal bodies are termed polembryoma. They are composed of an embryonal disc, yolk sac and amniotic cavities[7].

E) CHORIOCARCINOMA:

Primary ovarian choriocarcinoma are very rare. They usually present as metastases from the uterine tumours. They have better prognosis than non gestational choriocarcinoma[7].

Gross:

Pure choriocarcinoma present as solid, hemorrhagic and friable mass[7].

Microscopy:

Biphasic pattern of syncytial and cytotrophoblastic elements in necrotic and hemorrhagic back ground[7].

Immunohistochemistry for HCG (Human Chorionic Gonadotrophin) is positive.

F) TERATOMA:

Teratoma consists of tissues representing all the three germ layers. They are classified as

1. Immature teratoma
2. Mature teratoma
3. Monodermal teratoma[7].

1) IMMATURE TERATOMA:

It is a malignant entity. It constitutes of 1% of all ovarian tumours. The average age of presentation is around 20 years. The constituents have an embryonal appearance[7].

Gross:

They are mostly unilateral. Size ranges from 15 to 18cms. Cut surface shows partly cystic and solid areas. Focal areas of necrosis and hemorrhage are seen[44,45].

Microscopy:

A mixture of mature and immature elements are seen. Immature elements in the form of neuroepithelial tubules, rosettes, immature cartilage, fat, liver tissue, endodermal glands are seen.

The most widely used grading Norris *et al.,* grades the immature neuroepithelial elements based on amount of immature neuroepithelial elements[44,45].

Grading of immature teratoma:

Table 3:

Grade	Immature tissue	Amount of neuroepithelial tissue
1	+	Rare, Not > 1/LPF/Slide
2	+ +	Common, Not > 3/LPF/Slide
3	+ + +	Prominent > 4/LPF /Slide.

Grade 3 teratoma occasional present with glial masses, ependymal, neuroblastoma, medulloepithelioma like arrangements. Immature teratoma should be differentiated from

carcinosarcoma with heterologous elements. Immature teratoma is more common in younger age group and consists of neuroepithelial elements[7].

2) MATURE TERATOMA:

Most common benign tumour in western world is mature cystic teratoma. It accounts for 25% of all ovarian tumour. 90% of germ cell tumours present as mature cystic teratoma. More common in 20-50 years of age. Predominantly they are unilateral tumours (88%) [45,46].

Gross:

They present as multiloculated cyst. The cysts usually contain keratin, sebum, hair and teeth sometimes an imperfectly formed mandible or a partial human body like configuration (Homunculus/fetiform teratoma) is found. The characteristic Rokitansky protuberances with variety of tissue types are found. Solid areas should be grossed carefully to rule out immature teratoma[46].

Microscopy:

A mixture of ectodermal, mesodermal, endodermal, elements are seen. They are composed of hair follicles, epithelium, salivary gland, thyroid and respiratory tract epithelium. Rarely peritoneal glial implants/peritoneal melanosis are also seen[45,46].

Benign tumours include cutaneous adnexal tumours, salivary gland tumours. In post menopausal age group secondary malignancy can occur. Squamous cell carcinoma is the most common malignant tumour arising from it. Mature cystic teratoma can occur in association with mucinous cystadenoma, brenner and fibrothecoma.[(49)]

3) MONODERMAL TERATOMA:

It consists of tissues derived from one germ cell layer. The most common to occur is struma ovary. Other rare entities are carcinoid tumour and neuroectodermal elements tumour[7,16].

STRUMA OVARII:

The predominant component in this type is thyroid tissue. It constitutes 2 to 7% of all ovarian teratomas. Malignancy is rarely encountered. If it arises, it presents as papillary carcinoma with typical nuclear features. Grossly they are less than 10cms in maximum extension. Cut surface is solid tan with glistening surface. Microscopic examination shows numerous follicles filled with colloid[7,16].

CARCINOID TUMOUR:

The neuroendocrine tumours are more common in older age group. The patient present with menstrual irregularities and abdominal pain. Grossly they are unilateral. Cut surface shows tan yellow solid areas. Microscopically, the tumour cells are arranged in trabecular pattern. The nucleus has salt and pepper chromatin.

The most common site of metastases from intestinal carcinoid is ovary.The metastatic carcinoid present as multiple nodules and bilateral in presentation.

Immunohistochemistry markers like serotonin, chromogranin are positive[7,16].

G) MALIGNANT MIXED GERM CELL TUMOUR:

5 - 20% of malignant germ cell tumours belong to this category. Usually combination of dysgerminomas with yolk sac tumours can be seen. They are followed by immature teratoma, embryonal carcinoma, choriocarcinoma. The later group have grave prognosis. They are common in younger age group[7,16].

I) MIXED GERM CELL – SEX CORD STROMAL TUMOURS:

Dysgenetic gonadoma as it is otherwise called is associated with XY gonadal dysgenesis. They are composed of dysgerminoma and sex cord stromal tumours composed of immature sertoli and granulosa cells. Most commonly they are associated with hyalinization and calcification. The close differential diagnosis is sex cord tumours with annular tubules. The latter lacks germ cell components[7,16].

IV. TUMOURS OF RETE OVARII:

They are relatively uncommon tumours. They are seen in the postmenopausal women. The tumour is located in the ovarian hilus.

Microscopy:

Cuboidal cells, nonciliated columnar cells are seen arranged in retiform spaces. They may present as adenoma, cystadenoma, carcinoma[7].

V. TUMOURS OF UNCERTAIN ORIGIN:

A) SMALL CELL CARCINOMA:

They may present as

- Hypercalcemic type
- Pulmonary type.

HYPERCALCEMIC TYPE:

It is most common form of undifferentiated carcinoma. It is most common in young female and are always bilateral. The tumours are always associated with hypercalcemic. The above feature disappears after the removal of tumour.

Grossly they are large, solid with huge areas of necrosis and hemorrhage. Microscopically, small closely packed cells with high N/C ratio are seen. Numerous mitotic figures are seen. Because of extra ovarian spread, the prognosis is grim[35].

The pulmonary type resembles the lung tumour. They are sometimes associated with endometrial carcinoma[35]. Tumours are positive for keratin, EMA, NSE and rarely chromogranin[31].

B) TUMOURS OF PROBABLY WOLFFIAN ORIGIN:

Also called as wolffian adnexal tumour. They are benign tumours. Grossly they are solid, grey white or yellow in colour. Microscopically the epithelial cells are arranged in solid sheets with cystic degeneration. They are CK7, Calretinin, Vimentin positive[31].

VI) UNCLASSIFIED TUMOURS:

SARCOMAS:

Most of them are fibrosarcoma, leiomyosarcoma, endometrial sarcoma, osteosarcoma, chondrosarcoma, angiosarcoma, rhabdomyosarcoma. 8% of malignancy in dermoid cyst occur as sarcoma[32].

Fibrosarcoma:

Common in postmenopausal women. Grossly they are fleshy with areas of hemorrhage and necrosis. Histology shows spindle shaped cells arranged in Herringbone pattern. Nuclear atypia and mitotic figures are prominent[32].

VII) METASTATIC TUMOURS:

5 – 10% of ovarian tumours come under this category. The most common primary tumours to metastasize are large intestine, stomach, appendix. This is followed by breast, uterine corpus, uterine cervix.

In younger girls, metastasis occur in Neuroblastoma, Rhabdomyosarcoma, Ewing's sarcoma[13,48,49].

Features of ovarian metastasis:

1. Bilaterality
2. Surface involvement
3. Nodular pattern of growth
4. Hilar involvement
5. Single cell invasion
6. Desmoplastic reaction
7. Vascular invasion
8. Unusual extra ovarian spread.

The normal ovarian parenchyma is completely replaced. They appear as solid, white,

sometimes cystic. Multiple serosal implants is a diagnostic feature[48]. Immunohistochemistry is helpful in differentiating from primary which is CK7 positive of CK20 negative[31].

KRUKENBERG TUMOUR:

The most common primary site is stomach. Grossly, they are symmetrically/asymmetrically enlarged. Cut surface reveals predominantly homogenous with firm, white areas. Microscopically, the tumour cells are seen as isolated single cells, nests, cords. The individual cells have cytoplasmic vacuoles compressing hyperchromatic nucleus to one side. This gives a signet ring appearance. Abundant pools of mucin are seen in the stroma[50,51].

Special stains for mucin namely PAS and alcian blue and epithelial markers are positive[31]. This tumour has a grave prognosis[50].

PROGNOSIS OF OVARIAN TUMOURS:

The important factors influencing are as follows.

1. Histological type
2. Molecular abnormality
3. Stage of Ovarian cancer
4. Women's age and general health
5. Whether newly diagnosed/recurred[16]

ESTROGEN RECEPTOR:

Estrogen suppresses basal and cisplatin induced apoptosis. The etiopathogenesis is by increasing DNA repair capacity and avoiding apoptosis[56]. This leads to uncontrolled cell growth and drug resistance. The mechanism of action is by up regulation of c-myc gene. Estrogen also regulates genes (ezrin, fibulin, cathepsin D and kallikerins) involved in motility and invasion of extra cellular matrix[12].

PROGESTERONE RECEPTOR:

Progesterone promotes cell differentiation and apoptosis.Progesterone inhibits DNA synthesis and cell division. Many studies have shown that there is decreased risk of ovarian cancer with oral contraceptives and in multiparous individual. This could be due to cyclic progestational climate. (serum levels are comparable to luteal phase progesterone level)[2,6].

The mechanism of action is three fold . Firstly a connection exists between progesterone action and Fas/FasL signaling in normal and malignant ovarian surface epithelial cell death. Secondly, through decrease in membrane fluidity. Lastly, progesterone induces a switch from TGF-1 β_1 to TGF beta 2/3 expression. This correlated with increased apoptotic index in ovarian surface epithelium[2,11].

Her-2/neu RECEPTOR

The term neu was coined from the rat homologue of Her-2. The rats were induced to produce neuroblastoma by nitroso ethyl urea and their DNA was used for research purpose.[61]

The Her-2/neu oncogene is located on chromosome 17. Her-2/neu oncogene encodes a transmembrane glycoprotein tyrosine kinase. This includes four receptors-$ErbB_1$(HER), $ErbB_2$(HER-2/neu), $ErbB_3$(HER_3) and $ErbB_4$(HER_4). HER-2 expression is well documented in breast. It helps in assessment of prognosis (increased risk for early recurrence and resistance to endocrinopathy) and in the treatment of breast cancers. Targeted monoclonal antibody has been used a mode of treatment in breast cancers.[2,10,59]

Various studies have been shown that apart from breast, HER-2 over expression is also seen in stomach, prostate, ovary, colon and bladder. The four receptors are monomers. They dimerise when bound by a ligand either with same kind of receptor (homodimerisation) or with other receptor (heterodimerisation).

Receptors	:	Ligands
HER_1	:	EGF, amphiregulin, TGF (Transforming growth factor), epiregulin and betacellulin.
HER_2	:	$ASGP_2$ forming sialomucin complex, MUC_4 and phosphorylation of HER_2
HER_3, HER_4	:	Hergulins, acetylcholine receptor inducing activity, signals MAP(mitogen activated protein kinase path way).[61]

ROLE IN TUMORIGENESIS:

Activation of HER family of receptors causes activation of Ras, MAP (mitogen activated protein) kinase pathway and P13K (Phosphatidylinositol-3 Kinase) pathway. The RAS/MAP pathway stimulates cell proliferation. The PI3K pathway inhibits proapoptotic proteins like Bad, $GSK_3\beta$, FOXO3a through phosphorylation of AKt.[10,61]

HER_2 Over expression is being correlated to tumour size, grade, increased proportion of S phase cells and aneuploidy. HER_2/HER_3 heterodimer has a strong mitogenic effect in ovarian carcinoma. The combination of cytotoxic chemotherapy and EGFR/HER inhibitors (Trastuzumab) has a better clinical response.[10,59]

MASTER CHART

S.No.	HPE No.	Age in Years	Clinical Presentation	Gross features	HPE Diagnosis	FIGO Staging
1.	G20/13	28	Mass Abdomen	Unilateral, Cystic	Benign Mucinous Cystadenoma	I
2.	G29/13	35	Pain Abdomen	Unilateral, Cystic with Papillary excrescences.	Benign Serous cystadeno fibroma	I
3.	G46/13	30	Mass Abdomen	Unilateral, Cystic	Benign Mucinous cystadenoma	I
4.	G56/13	45	Mass Abdomen	Unilateral, Cystic	Benign mucinous cystadenoma	I
5.	G58/13	45	Pain Abdomen	Unilateral, Solid	Fibroma	I
6.	G70/13	30	Mass Abdomen	Unilateral, Cystic	Benign mucinous cystadenoma	I
7.	G76/13	30	Mass Abdomen	Unilateral, Cystic	Mature cystic Teratoma	I
8.	G79/13	35	Mass Abdomen	Unilateral, Solid and cystic	Granulosa-Theca cell Tumour	I
9.	G134/13	50	Mass Abdomen	Unilateral, Cystic	Benign Mucinous Cystadenoma	I
10.	G143/13	19	Mass Abdomen	Unilateral, Cystic with Papillary excrescences	Benign papillary Serous cystadeno fibroma	I
11.	G156/13	23	Mass Abdomen	Unilateral, Cystic	Mature cystic Teratoma	I
12.	G186/13	20	Pain Abdomen	Unilateral, Cystic	Mature cystic Teratoma	I

13.	G260/13	35	Mass Abdomen	Unilateral, Cystic	Mature cystic Teratoma	I
14.	G285/13	46	Mass Abdomen	Unilateral, Cystic	Mature cystic Teratoma	I
15.	G317/13	21	Mass Abdomen	Unilateral, Cystic	Mullerinosis	I
16.	G354/13	55	Mass Abdomen ascites.	Unilateral, Solid	Krukenberg Tumour	II
17.	G401/13	15	Pain Abdomen	Unilateral, Solid and cystic	Dysgerminoma	I
18.	G402/13	39	Mass Abdomen, ascites, Omental deposits	Unilateral, Solid, Variegated	Endometrioid Carcinoma grade-III	III
19.	G405/13	40	Mass Abdomen	Unilateral, Cystic	Mature cystic Teratoma	I
20.	G452/13	23	Pregnancy associated	Unilateral, Cystic	Benign mucinous cystadenoma	I
21.	G478/13	24	Mass Abdomen + deposits	Unilateral, Solid, Cystic, Variegated	Mixed germ cell Tumour. Dysgerminoma and Embryonal carcinoma.	III
22.	G520/13	55	Mass Abdomen	Unilateral, Cystic	Benign Serous cystadenoma	I
23.	G521/13	29	Mass Abdomen	Unilateral, Cystic	Benign Serous cystadenoma	I
24.	G542/13	48	Mass Abdomen	Unilateral, Cystic	Benign Serous cystadenoma	I
25.	G579/13	35	Mass Abdomen	Unilateral, Cystic	Endometrioid carcinoma	I
26.	G585/13	35	Mass Abdomen	Unilateral, Cystic	Benign Papillary Serous cystadenoma	I

27.	G588/13	55	Mass Abdomen	Unilateral, Cystic	Borderline Serous tumour	I
28.	G695/13	26	Pain Abdomen	Unilateral, Cystic	Mature cystic teratoma	I
29.	G700/13	50	Mass Abdomen	Unilateral, Cystic	Benign mucinous cystadenoma	I
30.	G708/13	33	Mass Abdomen	Bilateral, Cystic with papillary excrescences	Benign papillary Serous cystadenoma	I
31.	G751/13	50	Pain Abdomen	Unilateral, Cystic	Mature cystic teratoma	I
32.	G729/13	19	Mass Abdomen	Unilateral, solid and cystic with papillary excrescences	Mucinous cystadeno carcinoma	I
33.	G795/13	65	Mass Abdomen ascites	Bilateral, Cystic	Benign Serous cystadenoma	I
34.	G811/13	35	Mass Abdomen	Bilateral, Cystic	Benign mucinous cystdenoma	I
35.	G829/13	47	Mass Abdomen	Unilateral, Cystic	Benign mucinous cystdenoma	I
36.	G843/13	39	Mass Abdomen	Unilateral, Cystic	Benign mucinous cystdenoma	I
37.	G845/13	42	Mass Abdomen	Unilateral, Solid and Cystic	Benign Serous cystadenoma	I
38.	G851/13	53	Mass Abdomen	Unilateral, Solid and cystic with Hemorrhagic fluid	Fibro thecoma	I
39.	G853/13	60	Mass Abdomen ascites	Bilateral, cystic	Benign mucinous cystadenoma	I
40.	G857/13	33	Mass Abdomen	Unilateral, Cystic	Benign mucinous cystadenoma	I

41.	G872/13	60	Mass Abdomen	Unilateral, Cystic	Benign mucinous cystadenoma	I
42.	G911/13	52	Mass Abdomen	Unilateral, Cystic	Benign mucinous cystadenoma	I
43.	G918/13	32	Pain Abdomen	Unilateral, Cystic	Mature Cystic Teratoma	I
44.	G919/13	42	Mass, pain Abdomen	Unilateral, cystic with Hemorrhagic material	Twisted/congested Benign Mucinous cystadenoma	I
45.	G920/13	55	Mass Abdomen	Unilateral solid variegated	Endometrioid carcinoma grade III	II
46.	G925/13	45	Mass Abdomen	Unilateral solid cystic	Benign Serous cyst adeno fibroma	I
47.	G1021/13	21	Pain Abdomen	Bilateral, cystic	Bilateral mature cystic teratoma	I
48.	G1049/13	50	Mass Abdomen	Unilateral, solid, cyst with papillary excrescences	Endometrioid adeno carcinoma	II
49.	G1061/13	65	Mass Abdomen	Unilateral cystic with papillary excrescences	Borderline mucinous cystadenoma	I
50.	G1084/13	40	Mass Abdomen	Unilateral solid and cystic	Mucinous cyst adeno carcinoma	I
51.	G1123/13	20	Pregnancy associated	Unilateral, cystic	Mature Cystic Teratoma	I
52.	G1186/13	45	Mass Abdomen	Unilateral, solid cystic with papillary excrescences	Borderline Serous tumour	I
53.	G1195/13	60	Mass Abdomen	unilateral, solid with papillary excrescences	Papillary Serous cystadeno carcinoma	II
54.	G18/14	50	Mass Abdomen	unilateral solid and cystic	Papillary Serous cystadeno Carcinoma	I

55.	G57/14	60	Mass Abdomen ascites	Bilateral, solid and cystic	Papillary Serous cystadeno carcinoma	I
56.	G140/14	40	Mass Abdomen ascites	Unilateral, solid	Metastatic carcinomatous deposits	III
57.	G153/14	45	Mass Abdomen	Unilateral cyst with hemorrhagic material	Benign, congested mucinous cystadenoma	I
58.	G206/14	30	Pain abdomen	Unilateral, cystic	Mature cystic teratoma	I
59.	G233/14	23	Mass Abdomen	Unilateral, cystic	Mature Cystic Teratoma	I
60.	G238/14	23	Mass Abdomen	Unilateral, solid and cystic	Immature teratoma with gliomatosis peritonei	III
61.	G277/14	22	Mass Abdomen	Unilateral cystic with papillary excrescences	Benign papillary Serous cystadenoma	I
62.	G285/14	65	Mass Abdomen Ascites	Bilateral cystic with papillary excrescences	Bilateral Benign papillary Serous cystadenoma	I
63.	G300/14	25	Mass Abdomen	Unilateral, cystic	Benign Serous cystadenoma	I
64.	G323/14	16	Pain Abdomen	Unilateral, solid	Sclerosing stromal tumour	I
65.	G328/14	27	Mass Abdomen	Unilateral, cystic with papillary excrescences	Benign Serous cystadeno fibroma	I
66.	G333/14	21	Mass Abdomen	Unilateral cystic	Mature cystic Teratoma	I
67.	G335/14	17	Pain abdomen	Unilateral, cystic	Mature cystic Teratoma	I
68.	G344/14	35	Pain Abdomen	Unilateral cystic with hemorrhagic fluid	Benign Mucinous cystadenoma	I

69.	G348/14	40	Mass and Pain Abdomen	Unilateral cystic	Benign mucinous cystadenoma	I
70.	G399/14	40	Mass Abdomen	Unilateral solid and cystic	Borderline Mucinous Cystadenoma	I
71.	G436/14	35	Mass Abdomen	Unilateral, solid and cystic	Granulosa cell Tumour	II
72.	G438/14	40	Mass Abdomen	Unilateral and cystic	Mature cystic teratoma	I
73.	G445/14	46	Mass Abdomen	Bilateral, solid	Dysgerminoma High grade	I
74.	G458/14	42	Mass and Pain Abdomen	Unilateral, solid cystic with papillary excrescences	Papillary Serous cystadeno carcinoma	I
75.	G466/14	23	Pain Abdomen	Unilateral cystic	Mature cystic Teratoma	I
76.	G469/14	25	Mass Abdomen	Unilateral cystic and solid	Benign Mucinous cystadenoma	I
77.	G525/14	19	Mass, Pain Abdomen	Unilateral solid and cystic	Mucinous cystadeno carcinoma	I
78.	G530/14	50	Mass Abdomen	Unilateral cystic	Benign Serous cystadenoma	I
79.	G535/14	29	Mass Abdomen	Unilateral, cystic	Benign Mucinous cystadenoma	I
80.	G594/14	30	Mass and Pain Abdomen	Unilateral cystic with hemorrhagic fluid	Benign mucinous cyst adenoma	I
81.	G606/14	48	Mass Abdomen	Unilateral cystic and focal solid areas	Borderline mucinous cystadenoma	I
82.	G610/14	60	Mass Abdomen	Unilateral cystic	Benign Serous cystadenoma	I

83.	G619/14	26	Pain Abdomen	Unilateral cystic	Mature cystic Teratoma	I
84.	G621/14	65	Mass and Pain Abdomen	Unilateral cystic with hemorrhagic	Congested Benign Mucinous cystadenoma	I
85.	G644/14	29	Mass Abdomen	Unilateral cystic	Benign Serous cystadenoma	I
86.	G676/14	24	asymptomatic	Unilateral cystic	Benign Serous cystadenoma	I
87.	G754/14	50	Mass Abdomen	Unilateral cystic	Mature cystic teratoma	I
88.	G756/14	35	Mass Abdomen	Unilateral cystic	Mature cystic teratoma	I
89.	G771/14	60	Mass and Pain Abdomen	Unilateral cystic	Borderline mucinous cystadenoma	I
90.	G782/14	35	Mass Abdomen	Unilateral solid and cystic	Papillary Serous cystadeno carcinoma	II
91.	G798/14	52	Mass Abdomen	Unilateral cystic	Borderline mucinous cystadenoma	I
92.	G828/14	50	Mass Abdomen; ascites	Unilateral cystic	Benign mucinous cystadenoma	I
93.	G879/14	37	Mass Abdomen Pain	Unilateral, cystic with Hemorrhagic material	Congested Benign Serous cystadenoma	I
94.	G945/14	32	Mass Abdomen	Unilateral cystic with papillary excrescences	Benign papillary Serous cystadenoma	I
95.	G947/14	53	Mass Abdomen ascites	Unilateral cystic	Benign mucinous cystadenoma	I
96.	G973/14	70	Mass Abdomen	Unilateral solid	Fibro thecoma	I

97.	G983/14	22	Pain Abdomen	Unilateral cystic	Mature cystic Teratoma	I
98.	G1015/14	44	Mass Abdomen	Unilateral cystic	Benign mucinous cystadenoma	I
99.	G1003/14	44	Mass Abdomen	Unilateral, cystic	Mature cystic teratoma	I
100.	G1069/14	42	Mass Abdomen	Unilateral cystic with papillary excrescences and solid	Papillary Serous cystadeno carcinoma	I
101.	G1072/14	60	Mass and Pain Abdomen	Unilateral cystic	Mature cystic Teratoma	I
102.	G1077/14	40	Mass Abdomen ascites	Unilateral solid and cystic	Endometrioid adeno carcinomatous deposits	III
103.	G1083/14	57	Mass Abdomen	Bilateral solid, cystic with papillary excrescences	Borderline papillary Serous cystadeno carcinoma	II
104.	G1086/14	27	Mass Abdomen	Unilateral cystic	Benign Serous cystadenoma	I
105.	G1119/14	39	Pain Abdomen	Unilateral	Simple Serous cystadenoma	I
106.	G1124/14	55	Mass Abdomen	Unilateral solid	Fibroma	I
107.	G1126/14	30	Mass Abdomen	Unilateral cystic with papillary excrescences	Borderline Serous Tumour	I
108.	G1140/14	39	Mass, Pain Abdomen	Unilateral solid and cystic with papillary excrescences.	Benign papillary Serous cystadenoma	I
109.	G7/15	26	Mass and Pain Abdomen	Unilateral, cystic	Mature cystic Teratoma	I
110.	G15/15	65	Mass Abdomen	Unilateral, cystic	Benign Serous cystadenoma	I

111.	G18/15	63	Mass Abdomen ascites	Unilateral, cystic	Benign mucinous cystadenoma	I
112.	G34/15	26	Mass Abdomen	Unilateral, cystic with papillary excrescences	Benign papillary Serous cystadenoma	I
113.	G56/15	32	Mass Abdomen	Unilateral, cystic	Benign mucinous cystadenoma	I
114.	G72/15	24	Mass Abdomen	Bilateral, cystic	Bilateral mature cystic Teratoma	I
115.	G89/15	43	Mass Abdomen, chronic liver disease, splenomegaly	Bilateral, cystic	Benign mucinous cystadenoma	I
116.	G99/15	70	Mass Abdomen	Bilateral, solid cystic	Bilateral Benign Serous cystadenoma	II
117.	G120/15	53	Mass Abdomen	Unilateral solid cystic with papillary projections and Hemorrhagic areas and necrosis	Adult granulosa cell tumour	I
118.	G137/15	60	Mass Abdomen ascites	Unilocular cystic	Benign mucinous cystadenoma	I
119.	G143/15	45	Mass Abdomen	Unilateral cystic and solid	Benign mucinous cystadeno carcinoma	I
120.	G147/15	40	Mass Abdomen	Unilateral cystic	Borderline mucinous tumour of ovary	I
121.	G168/15	25	Mass Abdomen	Unilateral cystic and solid areas	Mature cystic teratoma	I
122.	G176/15	55	Mass Abdomen, ascites malignant cells	Unilateral, solid with papillary excrescences and Hemorrhagic	Serous adenocarcinoma	II
123.	G180/15	65	Mass Abdomen	Unilateral, cystic	Benign mucinous cystadenoma	I
124.	G218/15	40	Mass Abdomen	Unilateral cystic with Hemorrhagic fluid	Benign mucinous cystadenoma	I

125.	G310/15	30	Mass Abdomen	Unilateral cystic, solid	Benign Mucinous cystadenoma	I
126.	G313/15	24	Mass Abdomen	Unilateral cystic	Benign mucinous cystadenoma	I
127.	G322/15	50	Mass Abdomen	Unilateral cystic	Benign Serous cystadenoma	I
128.	G351/15	34	Mass Abdomen	Unilateral, cystic with papillary excrescences	Benign papillary Serous cystadenoma	I
129.	G355/15	55	Mass Abdomen ascites	Unilateral, cystic and solid with papillary excrescences omental deposits	High grade papillary Serous cystadeno carcinoma	III
130.	G363/15	45	Mass Abdomen	Unilateral, cystic	Benign Serous cystadenoma	I
131.	G365/15	15	Mass Abdomen	Unilateral, solid and cystic	Mature teratoma with Benign mucinous cystadenoma	III
132.	G389/15	39	Mass Abdomen	Unilateral cystic	Twisted cystic Teratoma	I

OBSERVATION AND RESULTS

This prospective study comprises 132 ovarian neoplasms. The cases were referred from Raja Mirasudhar Government Hospital (RMH), Thanjavur Medical College. The study period is from January 2013 to May 2015.

The following table shows the total number of ovarian neoplasms among all the female neoplasms.

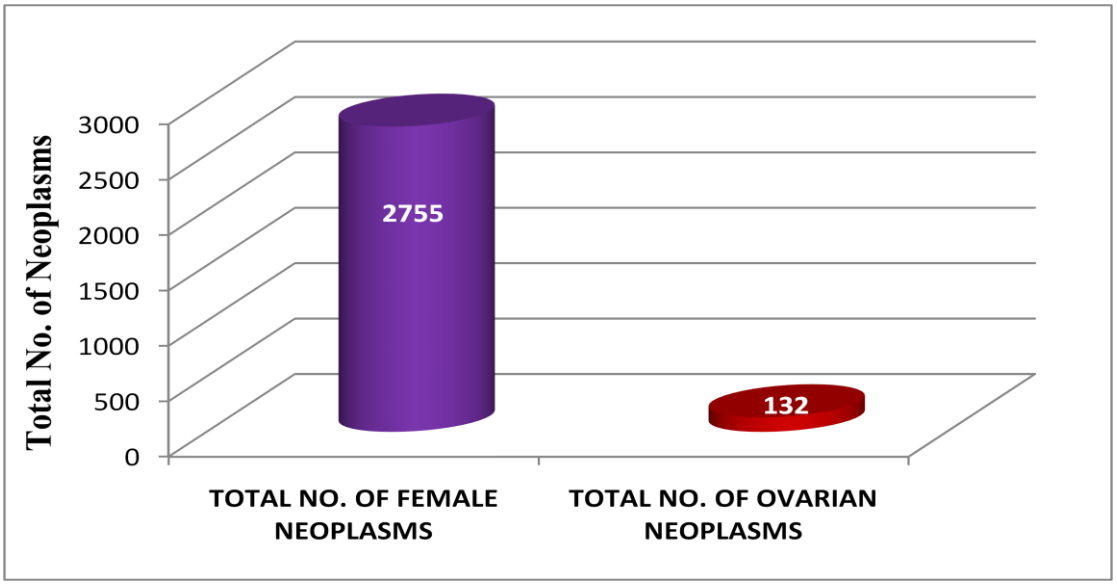
I. TOTAL NUMBER OF OVARIAN NEOPLASM IN COMPARISON WITH THE TOTAL FEMALE NEOPLASMS

Table 4:

S.NO.	PERIOD	TOTAL NO. OF FEMALE NEOPLASMS	TOTAL NO. OF OVARIAN NEOPLASMS	PERCENTAGE
1.	January 2013 – May 2013	438	19	4.3%
2.	June 2013-December 2013	761	33	4.3%
3.	January 2014 – May 2014	479	24	5.0%
4.	June 2014 – December 2014	676	32	4.7%
5.	January 2015 – May 2015	398	24	6.0%
	Total	**2755**	**132**	**4.7%**

The average incidence of ovarian neoplasm (Benign and malignant) among female is 4.7%

CHART 1: COMPARISION OF OVARIAN NEOPLASMS IN RELATION TO FEMALE NEOPLASMS



II. AGE INCIDENCE:

In this study, the age occurrence of ovarian tumours were ranging from 10 to 79 years. According to their age, the patients were divided into 7 groups (10-19 years, 20-29 years, 30-39 Years, 40-49 years, 50-59 years, 60-69 years, 70-79 years). The following table-5 shows the age incidence of ovarian neoplasms.

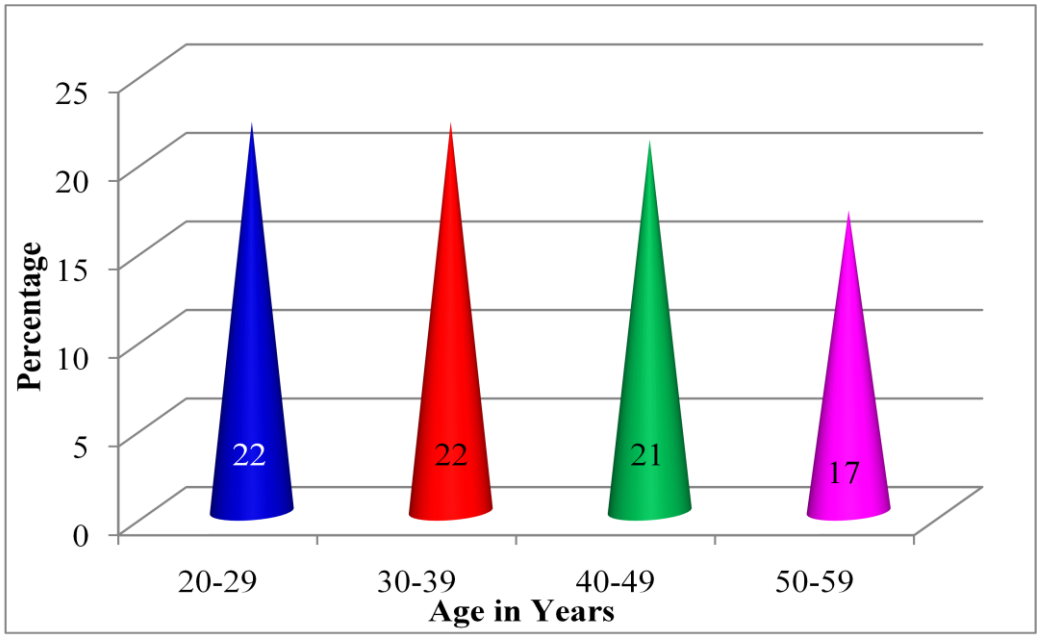
AGE INCIDENCE OF OVARIAN NEOPLASMS

Table 5:

S.NO.	AGE IN YEARS	TOTAL NO. OF CASES	PERCENTAGE
1.	10-19	7	5.3 %
2.	20-29	29	21.9 %
3.	30-39	29	21.9 %
4.	40-49	28	21.2 %
5.	50-59	22	16.6 %
6.	60-69	15	11.3 %
7.	70-79	2	1.5 %
	Total	**132**	

It is evident from the above table that the highest incidence is seen among the age group of 20-29 years and 30-39 years. After 70 years the incidence is lowest [CHART 2]

CHART 2: AGE INCIDENCE OF OVARIAN NEOPLASMS



III. AGE INCIDENCE OF CATEGORIES OF OVARIAN NEOPLASMS:

The ovarian neoplasms are classified into benign, borderline and malignant as given in the following table-6.

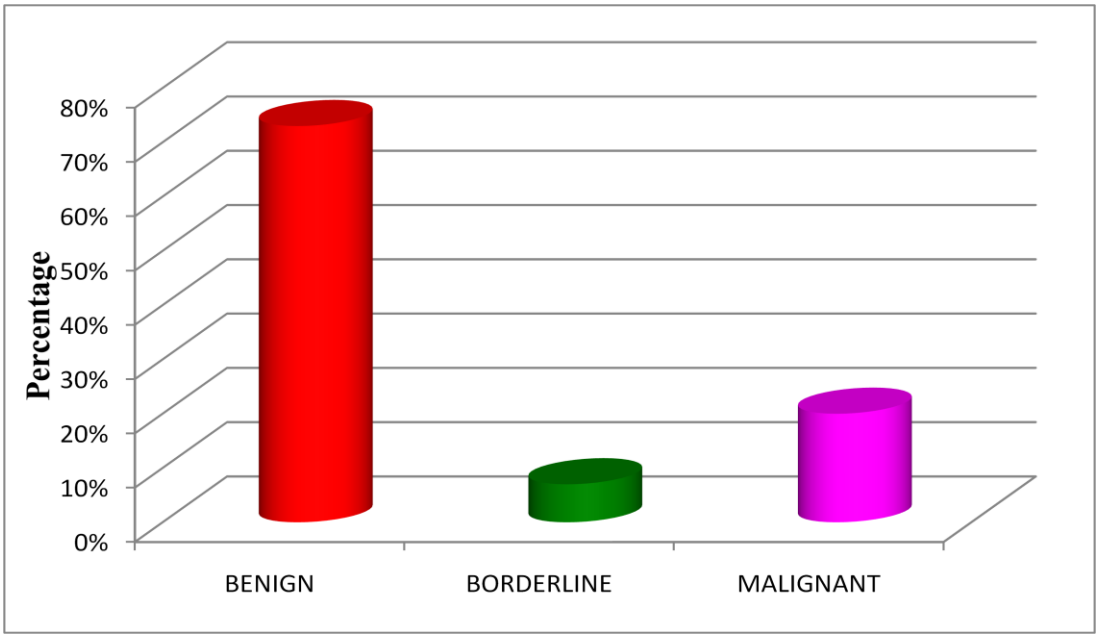
AGE INCIDENCE OF CATEGORIES OF OVARIAN NEOPLASMS

Table 6:

S.NO.	AGE IN YEARS	BENIGN	BORDERLINE	MALIGNANT
1.	10-19	4	-	3
2.	20-29	27	-	2
3.	30-39	24	1	4
4.	40-49	17	4	7
5.	50-59	12	2	8
6.	60-69	11	2	2
7.	70-79	2	-	-
	Total	97 73.4%	9 6.8%	26 19.6%

From the above table 6, The highest incidence of benign neoplasm is seen in the age group of 20-29 years (27/97 cases 27.8%). The Borderline tumours are common in the age group of 40-49 years (4/9 cases, 44.4%). The malignant tumours are common in the age group 50-59 years (8/26 cases, 30.7%) [CHART 3.]

CHART 3: AGE INCIDENCE OF CATEGORIES OF OVARIAN NEOPLASMS



FREQUENCY DISTRIBUTION OF INDIVIDUAL BENIGN TUMOURS IN DIFFERENT AGE GROUPS:

Table 6 A:

DIAGNOSIS	AGE IN YEARS						TOTAL %
	10-19	20-29	30-39	40-49	50-59	>60	
Serous cystadenoma	1	6	7	2	3	5	24 (18.1%)
Serous cystadeno fibroma	1	1	1	1			4 (3%)
Mucinous cystadenoma	0	6	11	8	5	6	36 (27.2%)
Fibroma				1	1		2 (1.5%)
Mature cystic teratoma	2	14	5	5	2	1	29 (21.9%)
Fibro thecoma					1	1	2(1.5%)
Total	**4**	**27**	**24**	**17**	**12**	**13**	**97 (100%)**

The benign tumours are common in the age group of 20-29 years (27/97 cases). They constitute 27.8% of the total number of benign ovarian neoplasms.

FREQUENCY DISTRIBUTION OF INDIVIDUAL MALIGNANT TUMOURS IN DIFFERENT AGE GROUPS:

Table 6 B:

DIAGNOSIS	AGE IN YEARS						TOTAL %
	10-19	20-29	30-39	40-49	50-59	>60	
Papillary Serous cystadeno carcinoma			1	2	4	2	9 (34.6%)
Mucinous cystadeno-carcinoma	2			2			4 (15.3%)
Granulosa cell tumour			1		1		2 (7.6%)
Dysgerminoma	1			1			2 (7.6%)
Endometrioid carcinoma			2	1	2		5(19.2%)
Mixed germ cell tumour		1					1 (3.8%)
Metastatic/krukenberg's tumours				1	1		2 (7.6%)
Immature teratoma		1					1 (3.8%)
Total	**3**	**2**	**4**	**7**	**8**	**2**	**26 (100%)**

From the above Table 6B, serous carcinomas are common in the age group 50-59 years (4/9 cases, 44.4%) Mucinous tumours are also common in the same group (2/4 cases, 50%) tumours are common after 40 years. In total, the malignant tumours are commonly seen in the postmenopausal age group of 50-59 years. They constitute about 8/26 cases, 30.7%

IV. CLINICAL EVALUATION:

The clinical features of the received cases were evaluated.

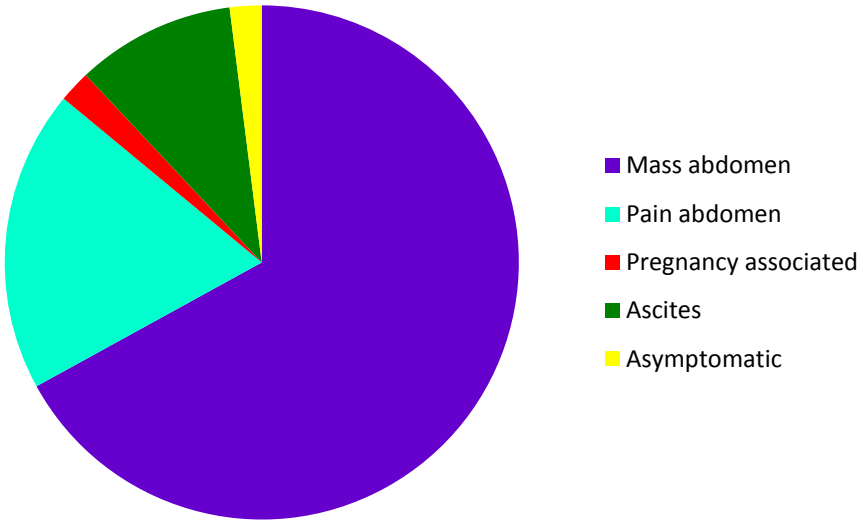
CLINICAL FEATURES OF VARIOUS OVARIAN TUMOURS:

Table 7:

S.NO.	CLINICAL FEATURES	NO. OF CASES	PERCENTAGE
1.	Mass abdomen	89	67.4%
2.	Pain abdomen	25	18.9%
3.	Pregnancy associated	2	1.5%
4.	Ascites	13	9.8%
5.	Asymptomatic	3	2.2%

Abdominal mass is the most common clinical presentation (89/132, 67.4%). Abdominal pain is the second most common clinical finding (25/132, 18.9%). 2 cases were found to be associated with pregnancy. 3 cases were asymptomatic and detected during abdominal ultrasonography done for other cases (CHART 4). 2 cases had positive metastatic deposits in the omentum. The ascitic fluid was positive for malignant cells in 2 cases.

CHART 4: PIE CHART DEPICTING THE PERCENTAGE OF SIGNS AND SYMPTOMS AMONG OVARIAN TUMOUR PATIENTS.



V. Laterality:

Neoplasms are subdivided as with unilateral/bilateral ovarian involvement as given table .8.

DISTRIBUTION OF OVARIAN TUMOURS IN RELATION TO LATERALITY

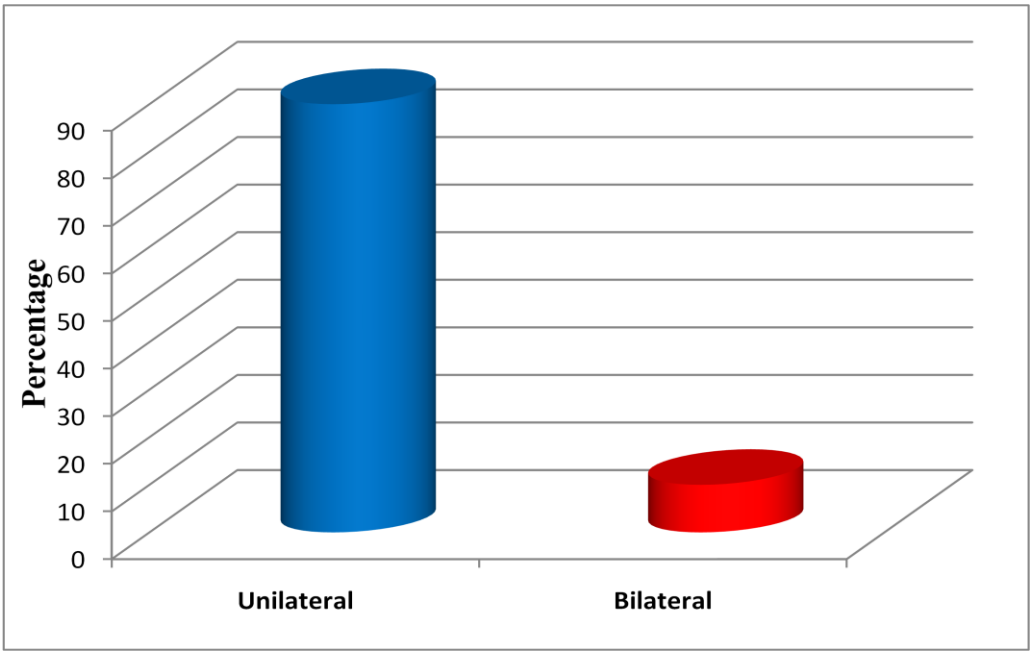
Table 8:

S.NO.	TUMOURS	UNILATERAL	PERCENTAGE	BILATERAL	PERCENTAGE
1.	**Serous** Benign Borderline Malignant	 24 3 7		 4 2	
	Total	34	28 %	6	54.5 %
2.	**Mucinous** Benign Borderline Malignant	 34 6 4		2	
	Total	44	36.3%	2	18.1 %
3.	Endometrioid	5	4.1 %		
4.	Sex cord stromal	8		1	
5.	Germ cell tumour	27	23 %	2	18.1 %
6.	Metastatic	1			
7.	Krukenberg's	1			
8.	Mixed germ cell tumour	1			
	Grand Total	**121**		**11**	

From the above table 8, it is evident that in serous tumours 34/40 cases (85%) were unilateral at the time of presentation and 15% were bilateral. Among mucinous tumours 44/46 cases (95.6%) were unilateral and 2/46 cases (4.3%) were bilateral.

Among germ cell tumour, 27/29 cases (93.1%) were unilateral and 6.8% were bilateral at the time of presentation.

CHART5: DISTRIBUTION OF OVARIAN TUMOURS IN RELATION TO LATERALITY OF INVOLVEMENT



Bilaterality is seen in 11 cases. In malignant neoplasms, most common neoplasm to occur bilaterally is serous tumours followed by the malignant germ cell tumours. None of the mucinous carcinomas were bilateral (chart -5).

VI. GROSS MORPHOLOGY OF OVARIAN NEOPLASM:

The ovarian tumours were classified into the following types based on gross morphology as shown in the following table 9.

GROSS MORPHOLOGY OF OVARIAN NEOPLASMS

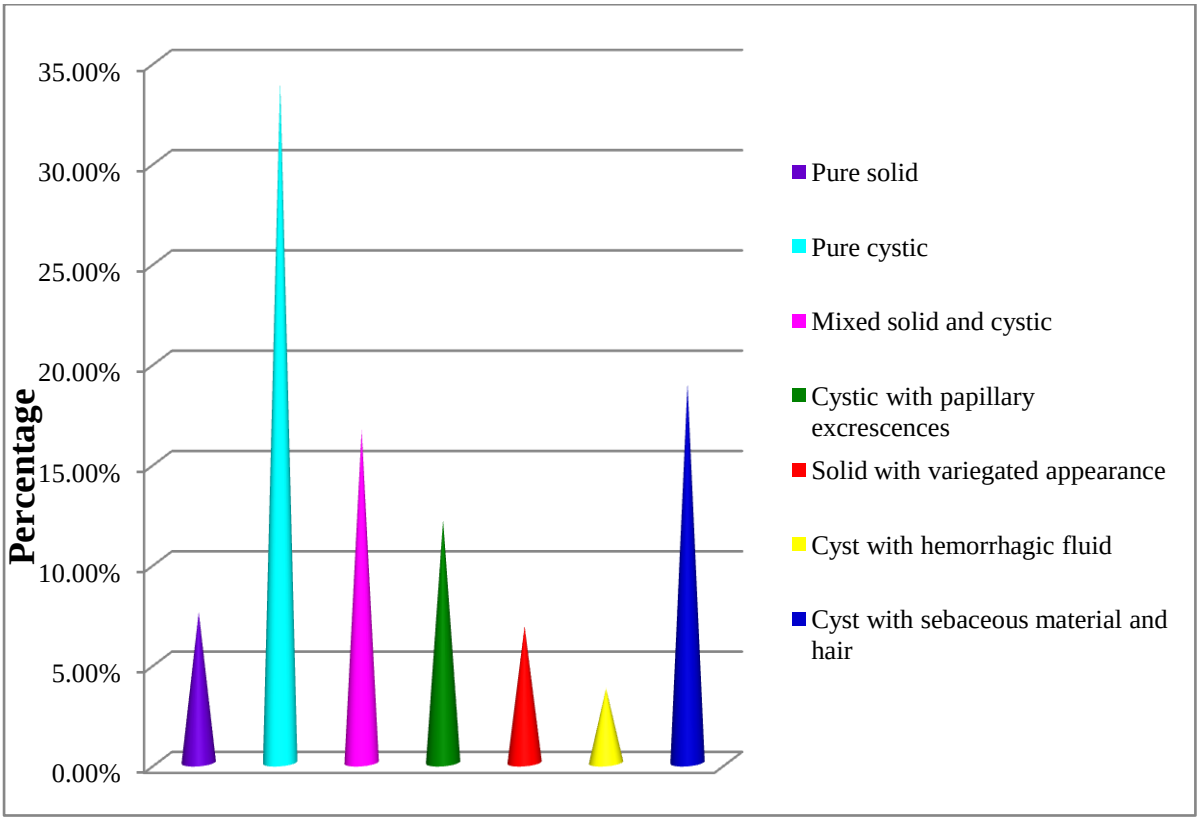
Table 9:

S. NO.	GROSS MORPHOLOGY	NUMBER OF CASES (%)
1.	Pure solid	10 (7.5%)
2.	Pure cystic	45 (34%)
3.	Mixed solid and cystic	22 (16.6%)
4.	Cystic with papillary excrescences	16 (12.1%)
5.	Solid with variegated appearance	9 (6.8%)
6.	Cyst with hemorrhagic fluid	5 (3.7%)
7.	Cyst with sebaceous material and hair	25 (18.9%)
	Total	**132 (100%)**

Ovarian neoplasms most commonly present as pure cystic masses 45 cases (34%). This is followed by cysts with sebaceous material and hair (25 cases, 18.9%) and mixed solid

and cystic tumours (22 cases 16.6%). The most uncommon presentation is solid with variegated appearance is 9 cases 6.8% (chart -6).

CHART 6: GROSS MORPHOLOGY OF OVARIAN TUMOURS



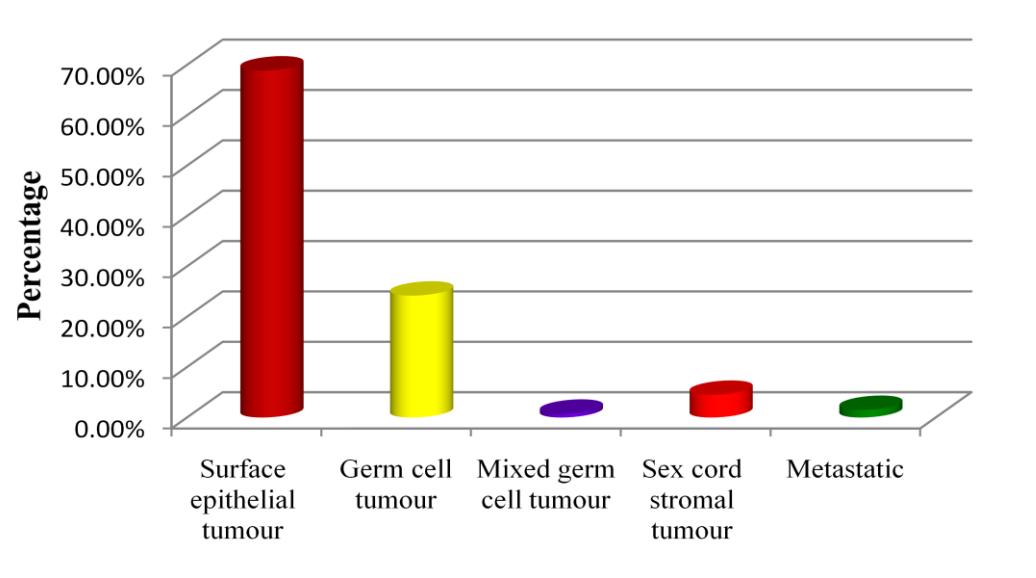
VII. DISTRIBUTION OF OVARIAN NEOPLASMS ACCORDING TO HISTOLOGICAL CLASSIFICATION:

Table 10:

S.NO.	CLASSIFICATION	NUMBER OF CASES	TOTAL CASES	PERCENTAGE
1.	**SURFACE EPITHELIAL TUMOUR** Benign Borderline Malignant	 64 9 18	91	68.9%
2.	**GERM CELL TUMOUR** Benign Malignant	 29 3	32	24.2%
3.	**MIXED GERM CELL TUMOUR**	1	1	0.75%
4.	**SEX CORD STROMAL TUMOUR** Benign Malignant	 4 2	6	4.5%
5.	**METASTATIC**	2	2	1.5%
	Total	132	132	

The above table, signifies out of 132 neoplasms surface epithelial tumour is the most common (91 cases, 68.9%) followed by germ cell tumours (32 cases, 24.2%) and sex cord stromal tumours(6 cases, 4.5%) (CHART 7)

CHART 7: INCIDENCE OF HISTOLOGICAL TYPES OF OVARIAN NEOPLASMS IN RELATION TO TOTAL OVARIAN NEOPLASMS



VIII. SUB CLASSIFICATION OF SURFACE EPITHELIAL TUMOURS:

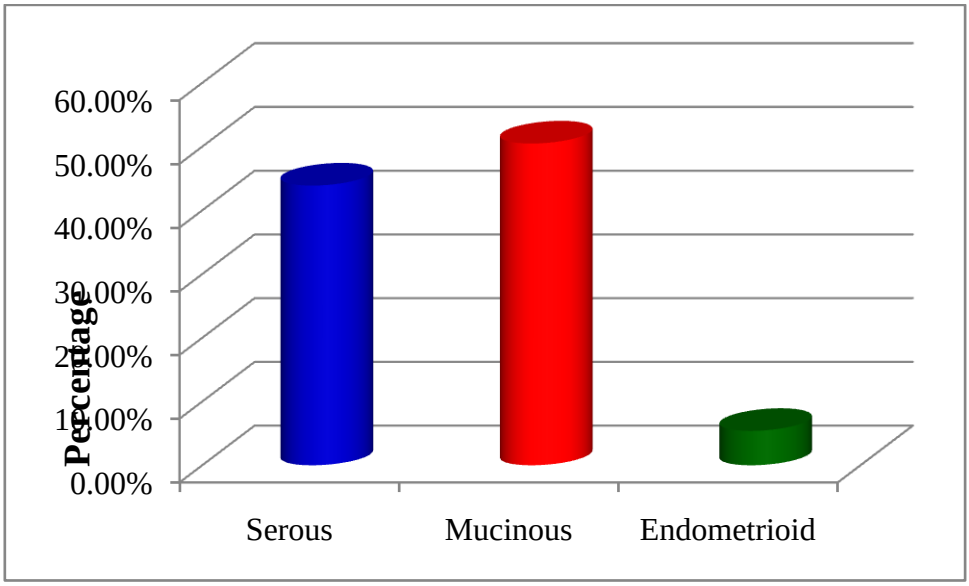
The Surface epithelial tumours are classified according to WHO HISTOLOGICAL CLASSIFICATION and is as follows in table 11.

Table 11:

S.NO.	CLASSIFICATION	NUMBER OF CASES	PERCENTAGE	AVERAGE
1.	**SEROUS TUMOURS** Benign Borderline Malignant	 28 3 9	 21.2% 2.2% 6.8%	43.9%
	TOTAL	40		
2.	**MUCINOUS** Benign Borderline Malignant	 36 6 4	 27.2% 4.5% 3%	50.5%
	TOTAL	46		
3.	**ENDOMETRIOID MALIGNANT**	5	3.7%	5.4%
	GRAND TOTAL	91		

From the above table 11, in surface epithelial tumours, in our institution, mucinous tumours predominates (46 cases, 50.5%) followed by serous tumours (40 cases, 43.9%). 9 cases of serous cystodenocarcinomas and 4 cases of mucinous cystadenocarcinomas were reported. Also 5 cases of Endometrioid carcinomas were reported. (CHART 8).

CHART 8: INCIDENCE OF HISTOLOGICAL SUB TYPES OF SURFACE EPITHELIAL TUMOURS



IX. HISTOMORPHOLOGICAL FEATURES OF BORDERLINE SURFACE EPITHELIAL TUMOURS:

Table 12:

S.NO.	HISTOLOGICAL FEATURE	NUMBER OF CASES
1.Serous Borderline	Histology	
	1. Typical	2
	2. Micropapillary	1
	Laterality	
	1. Unilateral	3
	2. Bilateral	0
	Surface involvement	
	1. Present	0
	2. Absent	3
	Microinvasion	
	1. Present	0
	2. Absent	3
2.Mucinous Borderline	Histology	
	1. Intestinal	5
	2. Endocervical	1

The above table 12 depicts that predominantly borderline serous tumours presented with hierarchical branching pattern of papillae (2/3 cases, 66.6%) with all the cases being unilateral at presentation. None of the cases had evidence of surface involvement or microinvasion. The mucinous tumours were predominantly of intestinal type (5/6 cases, 83.3%)

X. HISTOMORPHOLOGY OF MALIGNANT SURFACE EPITHELIAL TUMOUR:

Table 13:

S.NO.	HISTOLOGICAL TYPE	NUMBER OF CASES
1.	Serous Grading	
	1. Low Grade	3
	2. High Grade	6
	Total	**9**
2.	Mucinous Type of invasion	
	1. Expansive	4
	2. Infiltrating	0
	Total	**4**

Malignant serous neoplasms were graded according to recent 2 tier system of classification. High grade tumours exhibited marked nuclear atypia and >12 mitosis/10Hpf. From the above table 13, 3/9 cases (33.3%) were low grade carcinomas and 6/9 cases (66.6%) were high grade carcinomas. High grade carcinomas constitute the commonest type in the malignant serous tumours. All the 4 cases of mucinous carcinomas presented with expansile type of invasion with back to back gland arrangement and no stroma in between.

XI. SEX CORD STROMAL CELL TUMOURS:

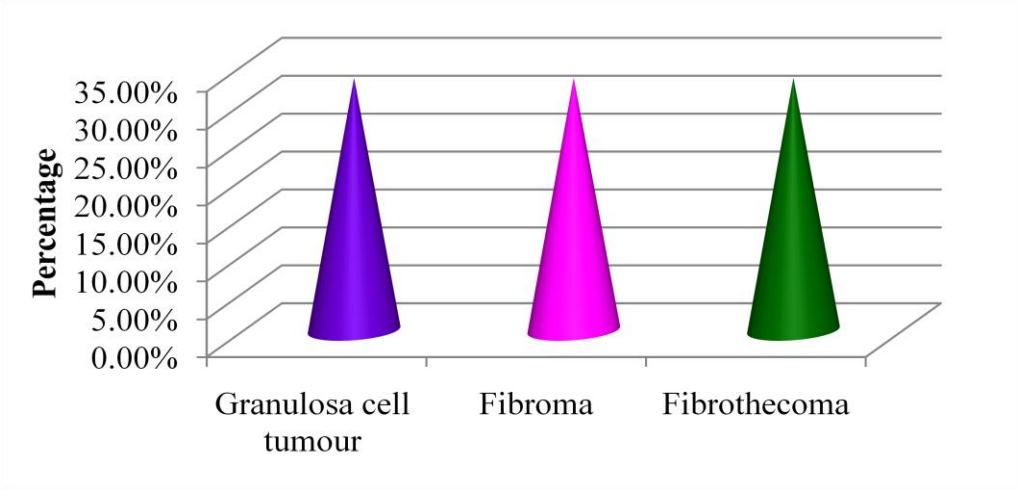
The sex cord stromal tumours were sub classified and the incidence is as follows.

Table 14:

S.NO.	CLASSIFICATION	NUMBER OF CASES	PERCENTAGE
1.	Granulosa cell tumour (adult type)	2	33.3%
2.	Fibroma	2	33.3%
3.	Fibrothecoma	2	33.3%
	Total	**6**	

From the above table, in sex cord stromal cell tumours granulosa cell tumour, fibroma and fibrothecoma had the same incidence of (2/6 cases, 33.3%) (CHART 9). All the cases reported as granulose cell tumour were adult type.

CHART 9: INCIDENCE OF SUB TYPES OF SEX CORD STROMAL TUMOURS



XII. GERM CELL TUMOURS:

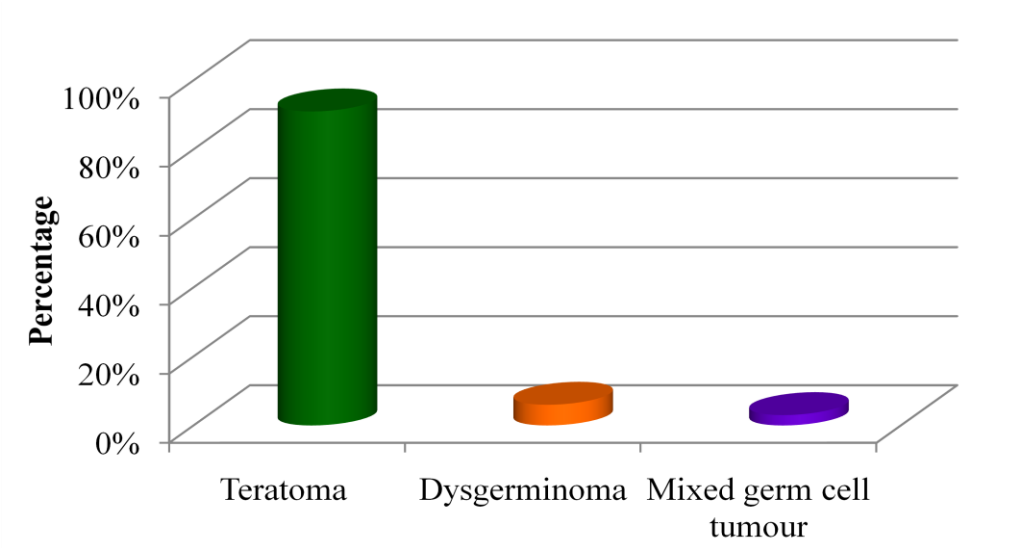
Germ cell neoplasms were subdivided and their individual type incidence is as given in the following table 15.

Table 15:

S.NO.	CLASSIFICATION	NUMBER OF CASES	PERCENTAGE
1.	TERATOMA 1. Benign 2. Malignant	29 1	90.9%
2.	Dysgerminoma	2	6%
3.	Mixed germ cell tumour	1	3%
	Total	**33**	

The above table depicts that among germ cell tumours, mature cystic teratomas dominates (29 cases, 90.9%) followed by dysgerminoma (2 cases 6%) and mixed germ cell tumour (1 case, 3%) (CHART 10). The mixed germ cell tumour was a combination of dysgerminoma and embryonal carcinoma.

CHART 10: INCIDENCE OF SUB TYPES OF GERM CELL TUMOURS



XIII. IMMUNOHISTOCHEMISTRY:

EXPRESSION PROFILE OF ER, PR AND Her-2/neu IN MALIGNANT TUMOURS.

Table 16:

Sl. No.	HPE No.	HPE DIAGNOSIS	ER	PR	Her-2/neu
1	579/13	Endometrioid carcinoma	Negative	Negative	Negative(1+)
2	920/13	Endometrioid carcinoma	Positive	Positive	Negative(0+)
3	1077/14	Endometrioid carcinoma	Strong Positive	Strong Positive	Negative(0+)
4	1195/13	High grade serous carcinoma	Positive	Positive	Negative(1+)
5	57/14	Bilateral High grade serous carcinoma	Positive	Negative	Positive(3+)
6	458/14	High grade serous carcinoma	Negative	Positive	Positive(3+)
7	782/14	Low grade serous carcinoma	Negative	Negative	Negative(0+)
8	355/14	High grade serous carcinoma	Positive	Positive	Positive(3+)
9	792/13	Mucinous carcinoma	Negative	Negative	Negative(0+)
10	1084/13	Mucinous carcinoma	Negative	Negative	Positive(2+)
11	525/14	Mucinous carcinoma	Negative	Negative	Negative(1+)

11 cases out of 26 malignant surface epithelial ovarian tumors were selected for immunohistochemistry. ER, PR expression was evaluated in the 11 malignant surface epithelial ovarian cancers.

ER and PR expression was based on the proportion of cells in a given tumor specimen exhibiting distinct nuclear immune positivity as well as intensity of staining (Negative <10% and positive ≥10%) (Table 16).

11malignant surface epithelial ovarian including carcinomas of different grades were taken for Her-2/neu analysis. Her-2/neu grading 0 and 1+ were considered as negative and 3+ as positive, 2+ was considered to be equivocal. From the above Table 16, we infer ER, PR expression is stronger in Endometrioid (2/3cases, 66.6%) and serous (3/5 cases, 80%). They had negative expression in mucinous tumours.

Her-2/neu expression is higher in malignant tumour when compared to borderline tumours (Table 17). And in histological types Her-2/neu expression is higher in high grade serous carcinoma when compared to low grade tumours, mucinous and Endometrioid carcinomas.

XIV. EXPRESSION PROFILE OF Her-2/neu IN BORDERLINE TUMOURS.

Table 17:

Sl. No.	HPE No.	HPE DIAGNOSIS	Her-2/neu
1	1126/14	Borderline serous tumour	Negative(1+)
2	G1061/13	Borderline mucinous tumour	Negative(0+)
3	G399/14	Borderline mucinous tumour	Negative(0+)
4	G798/14	Borderline mucinous tumour	Negative(0+)
5	G77/14	Borderline mucinous tumour	Negative(0+)

5 out of 9 cases were selected for immunohistochemical analysis. The expression of Her-2/neu in 2 types of borderline surface epithelial tumour were studied and the expression is tabulated as above (Table 17). All the Borderline tumours showed negative expression of Her-2/neu.

XV. EXPRESSION ER, PR in SEROUS TUMOURS.

Table 18:

Type of serous tumour	No. of cases	ER Expression	PR Expression
High Grade	4	Positive	Positive
Low Grade	1	Negative	Negative

The above Table 18 shows that the high grade serous tumour were positive for ER, PR receptors. The low grade serous tumours are negative for ER, PR receptors

XVI. EXPRESSION OF ER, PR IN MUCINOUS TUMOURS:

From the Table 16, it is evident that all the mucinous tumours in our study showed negative expression for ER and PR Receptors.

XVII. EXPRESSION OF ER, PR IN ENDOMETRIOID TUMOURS:

From the Table 16, we infer that all the three Endometrioid tumours showed positive expression of ER and PR Receptors. Based on four tiered scale (Level 1, <10%, Level 2, 10-40%, Level 3, 50-70% and Level 4, >80%) of cells showing positive expression, One case showed Level 4, i.e., >80% expression of both ER and PR expression.

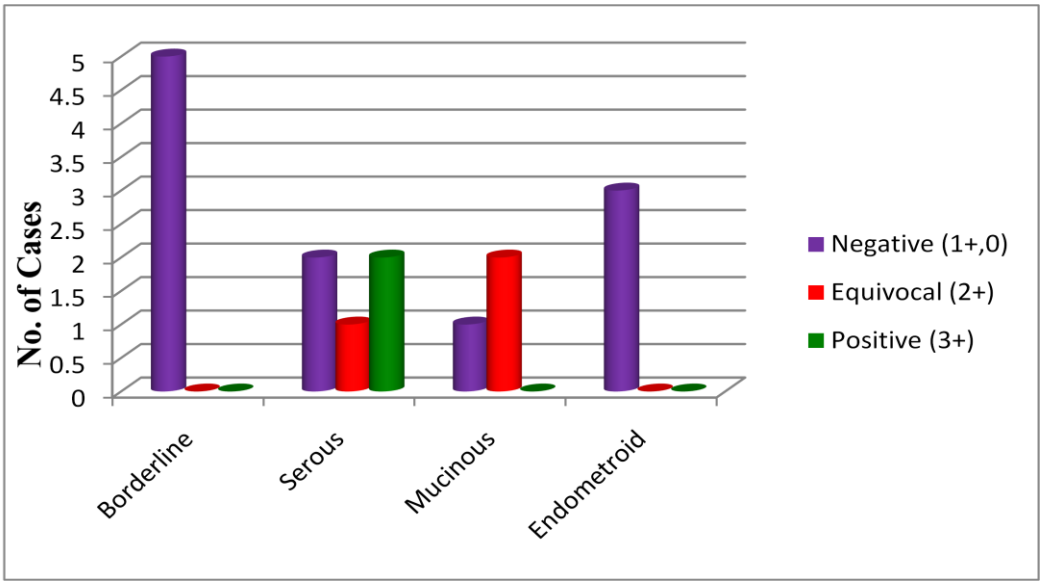
XVIII. EXPRESSION OF Her-2/neu IN BORDERLINE AND MALIGNANT SURFACE EPITHELIAL OVARIAN TUMOURS:

Table 19:

S.No.	Type of Tumour	No. of cases	Positive (3+)	Equivocal (2+)	Negative (1+, 0)
1	Borderline Tumours	5	-	-	5
2	Serous Tumours High Grade	5	2		2
3	Serous Tumours Low Grade		-	1	-
4	Mucinous Tumours	3	-	2	1
5	Endometrioid Tumours	3	-	-	3

From the above table it is evident Her-2/neu expression in the borderline tumour is totally negative. In malignant category 2/11 cases (18.1%) showed (3+) positive immunostaining. 3/11 cases (27.2%) were equivocal(2+). 6/11 (54.5%) showed negative expression. It is inferred from the above table Her-2/neu expression is significantly positive in malignant tumour, Her-2/neu expression was associated with high grade surface epithelial ovarian tumours. However, the intensity of positivity did not correlate with the type or grade of the tumour.

CHART 11: EXPRESSION OF Her-2/neu IN BORDERLINE AND MALIGNANT SURFACE EPITHELIAL OVARIAN TUMOURS



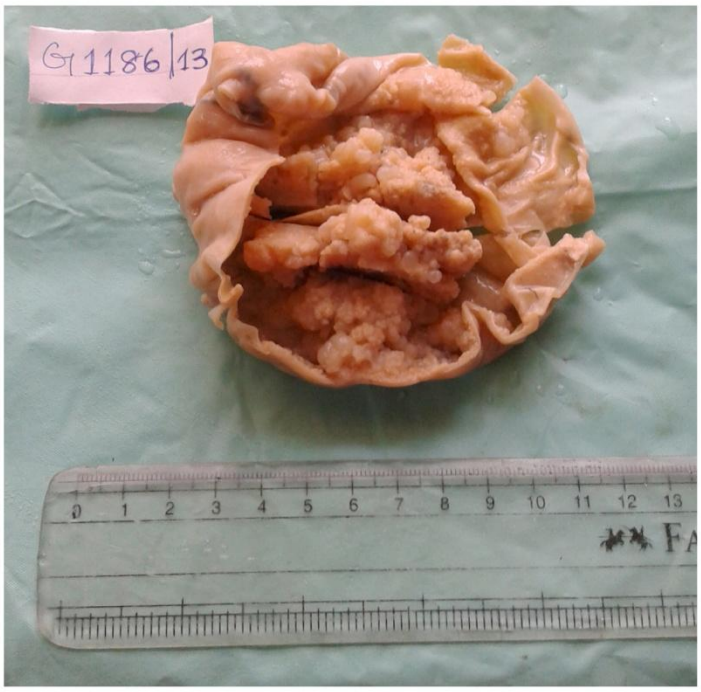


Figure.1 : BORDERLINE SEROUS CYSTADENOMA. CUTSURFACE MULTI LOCULATED CYST WITH PAPILLARY EXCRESENCES

Figure.2 :BILATERAL PAPILLARY SEROUS CYSTADENOCARCINOMA WITH SOLID AND CYSTIC AREAS

Figure.3 : MUCINOUS CYSTADENOCARCINOMA. CUT SURFACE- MULTILOCULATED CYST FILLED WITH MUCIN AND SOLID AREAS

Figure.4 : ENDOMETRIOID CARCINOMA - METASTATIC CARCINOMATOUS DEPOSITS FROM ENDOMETRIUM

Figure.5 : FIBROTHECOMA.CUTSURFACE-SOLID, HOMOGENOUS, FIRM, YELLOW AREAS

Figure.6 : IMMATURE TERATOMA WITH GLIOMATOSIS PERITONEI. CUTSURFACE-SOLID AND CYSTIC WITH FOCAL GLISTENING AREAS, AND IMPLANTS IN PERITONIUM.

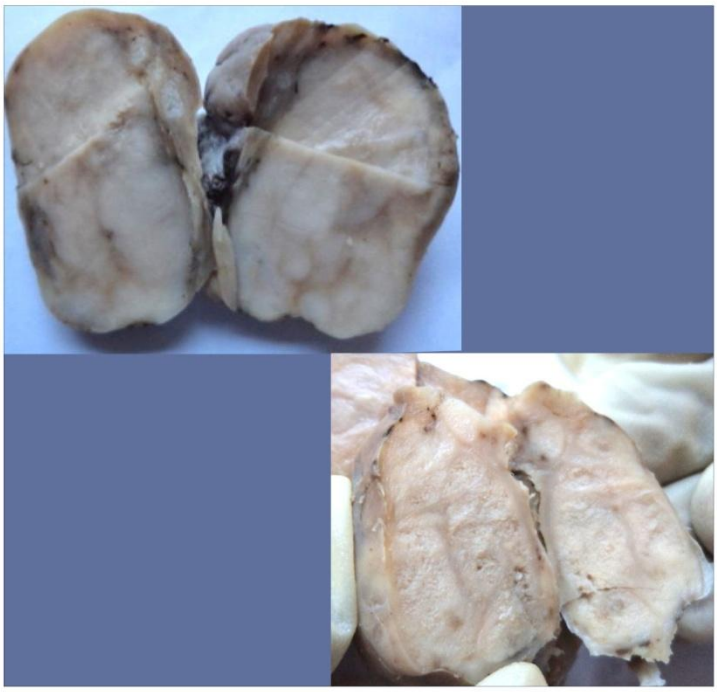


Figure.7A :BILATERAL DYSGERMINOMA.CUTSURFACE-SOLID, HOMOGENOUS

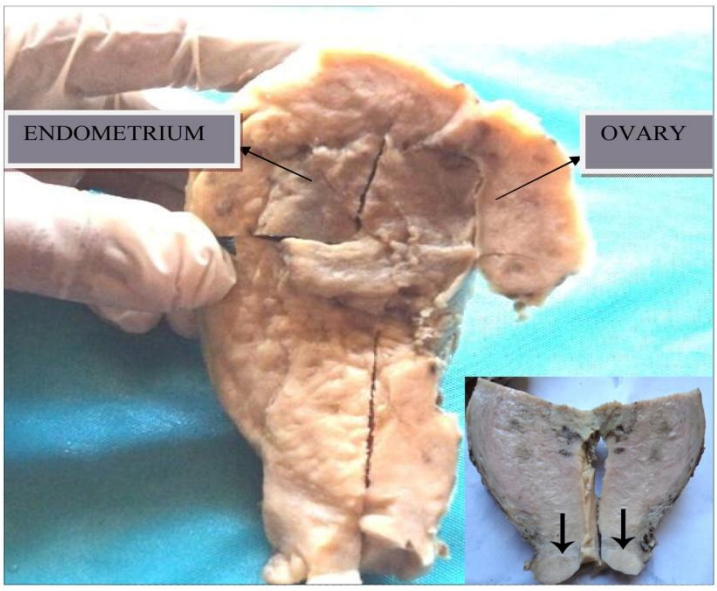


Figure.7B : BILATERAL DYSGERMINOMA WITH METASTASIS IN ENDOMETRIUM AND CERVIX

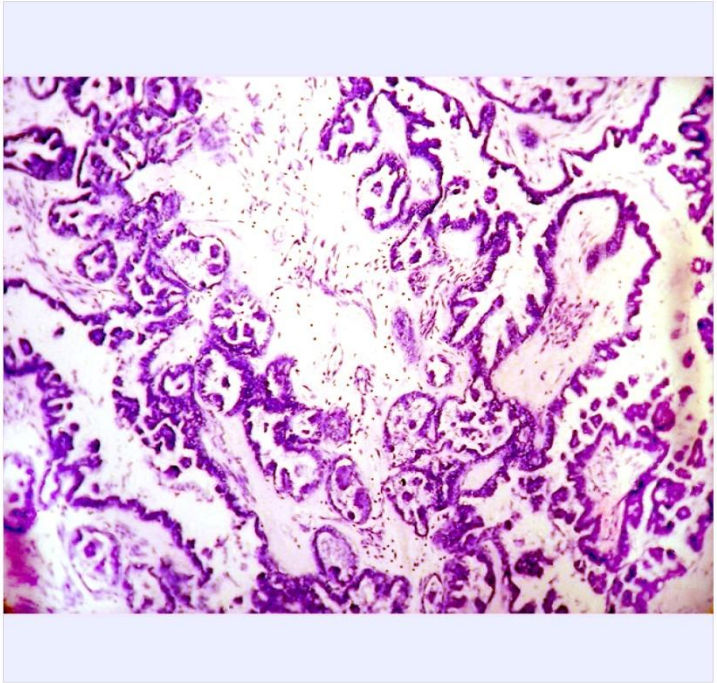


Figure.8 : SEROUS BORDERLINE TUMOUR WITH PAPILLARY FRONDS LINED BY CELLS WITH MILD TO MODERATE ATYPIA WITHOUT STROMAL INVASION (10X)

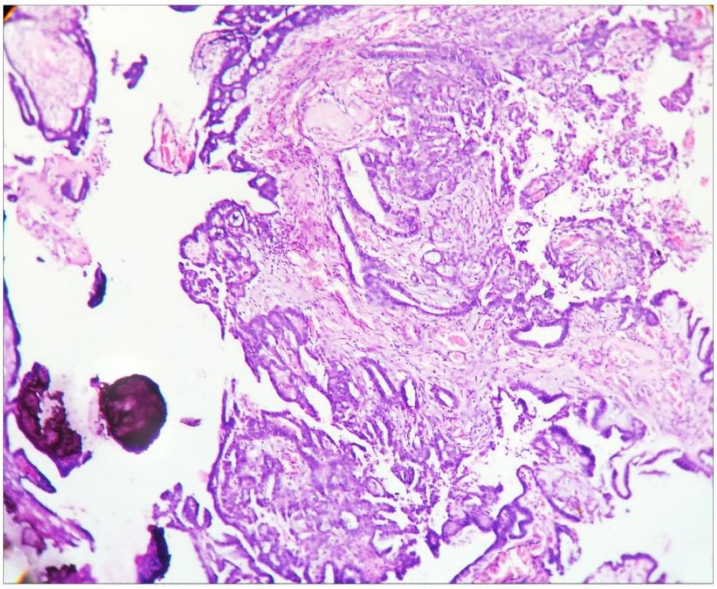


Figure.9 : LOW GRADE SEROUS CARCINOMA SHOWING PAPILLARY FRONDS LINED BY CELLS WITH MILD TO MODERATE ATYPIA (10X)

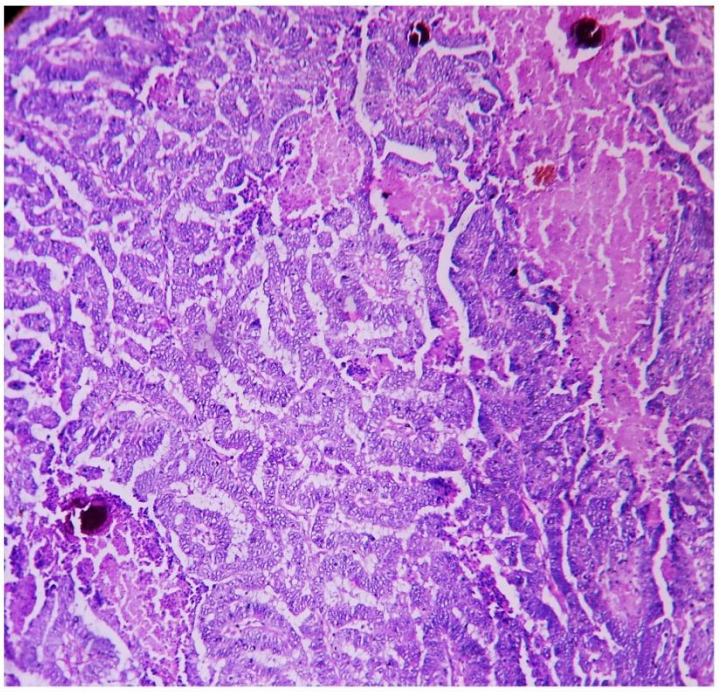


Figure.10 : HIGH GRADE SEROUS CARCINOMA WITH SOLID PROLIFERATION OF TUMOUR CELLS AND GLANDULAR ARCHITECTURE (40X)

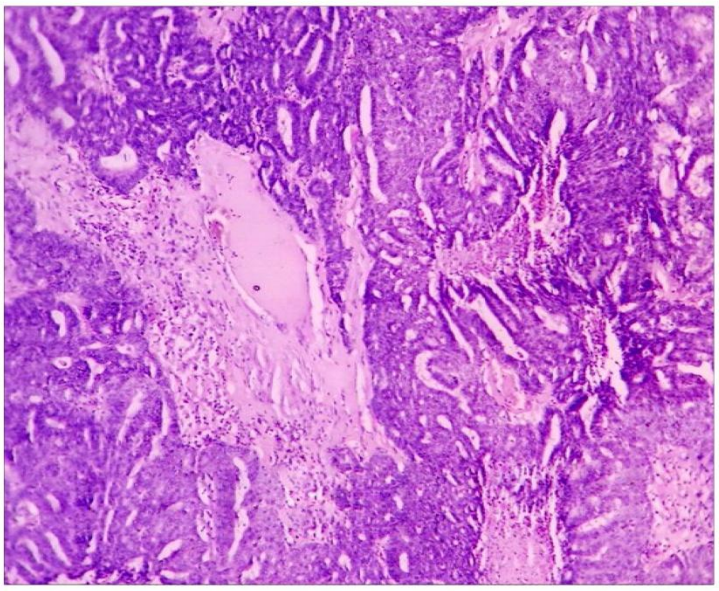


Figure.11 : HIGH GRADE ENDOMETRIOID CARCINOMA SHOWING BACK TO BACK GLAND ARRANGEMENT (40X)

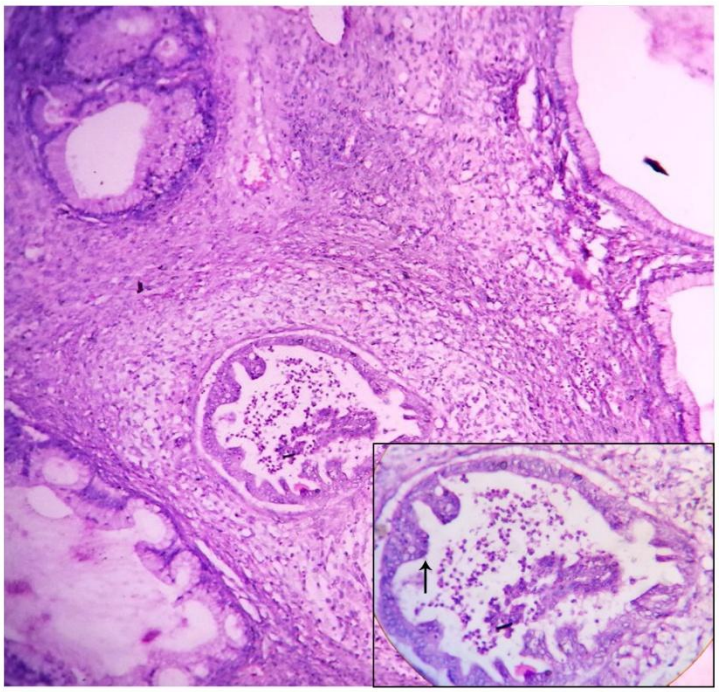


Figure.12 : MUCINOUS BORDERLINE TUMOUR-INTESTINAL TYPE, HIGH POWER VIEW SHOWING GOBLETCELLS AND STRATIFICATION

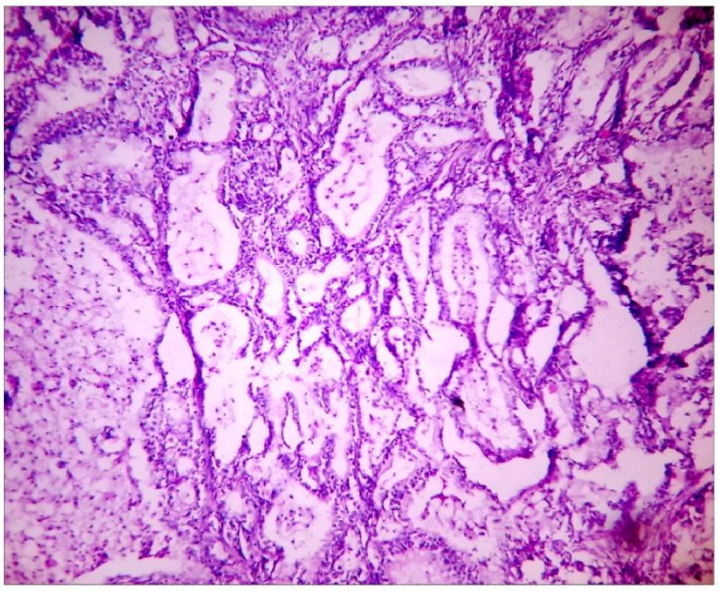


Figure.13 : MUCINOUS CARCINOMA SHOWING EXPANSILE INVASION - CLOSELY PACKED BACK TO BACK GLANDS (10X)

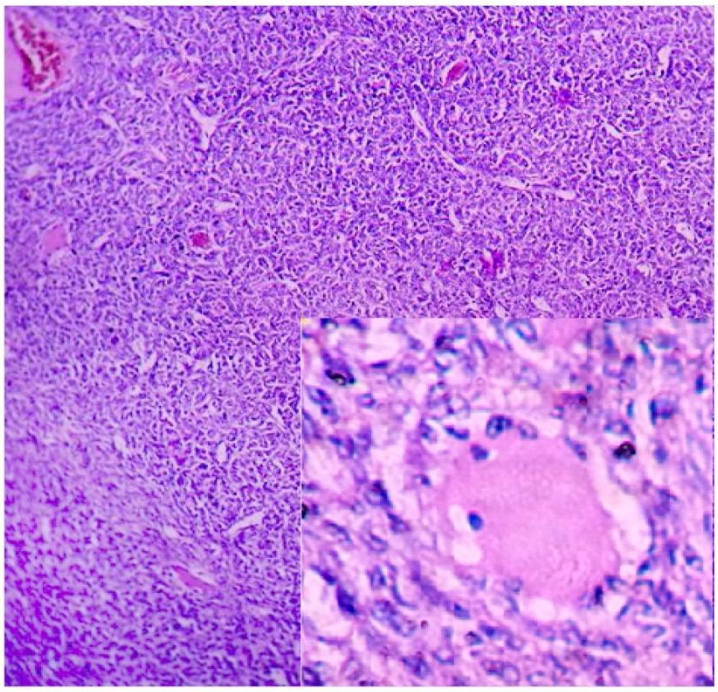


Figure.14 : WELL DIFEERENTIATED GRANULOSA CELL TUMOUR WITH MICROFOLLICULAR PATTERN (10X) NUMEROUS CALL EXNER BODIES CONTAINING HYALINE MATERIAL (40X)

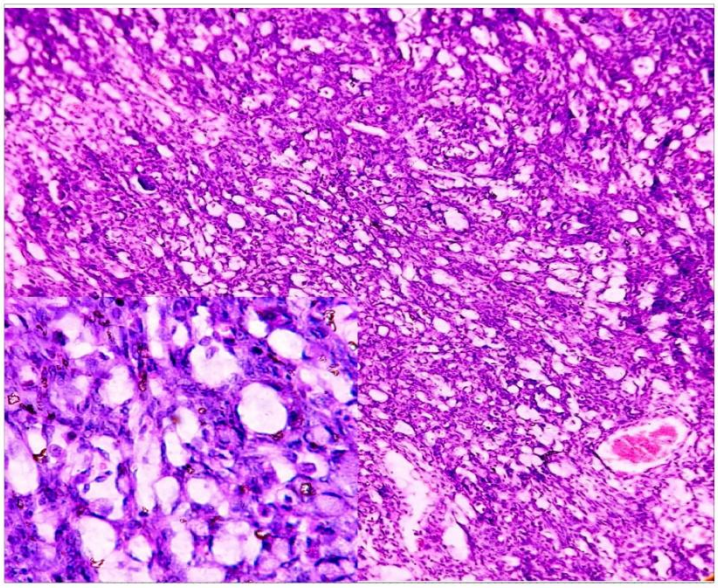


Figure.15 : KRUKENBERG'S TUMOUR - NUMEROUS SIGNET RING CELLS PRESENT IN HIGHLY FIBROUS STROMA

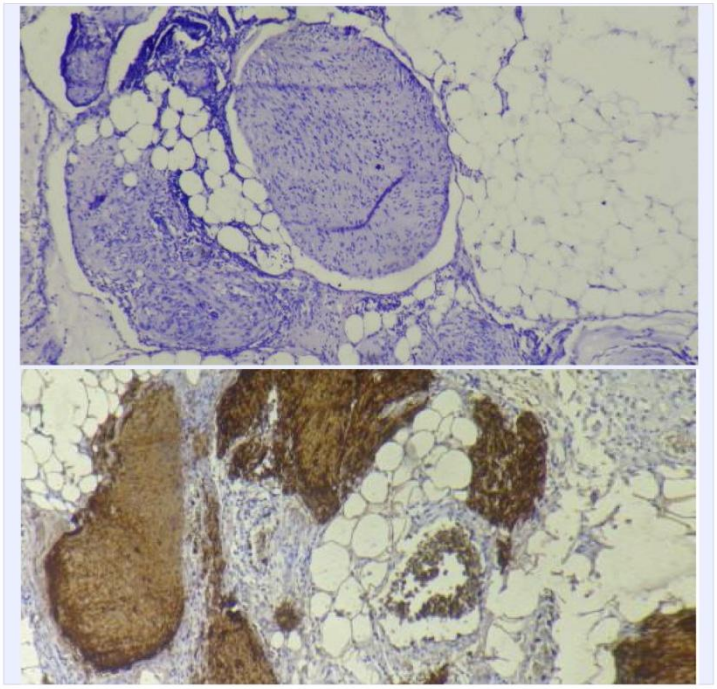


Figure.16 : GLIOMATOSIS PERITONEI - OMENTAL DEPOSITS, GFAP EXPRESSION

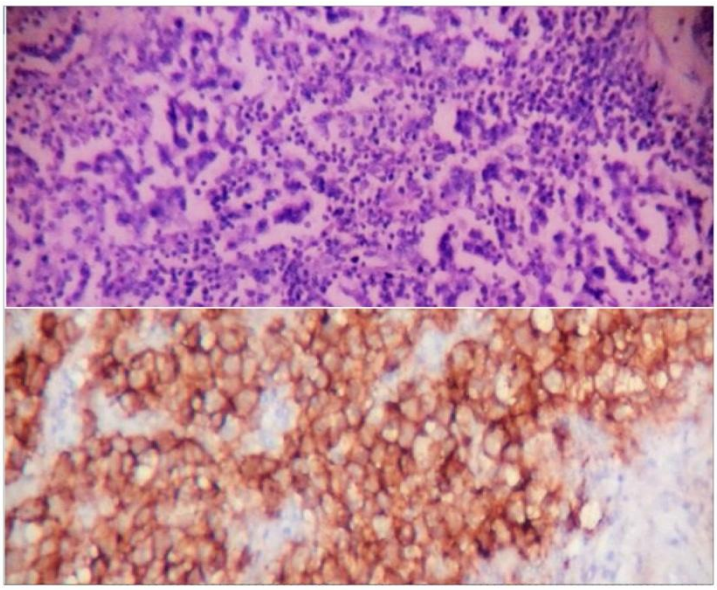


Figure.17 : DYSGERMINOMA - POLYGONAL CELLS WITH WATERY CLEAR CYTOPLASM, CENTRAL NUCLEUS, CDII7 EXPRESSION

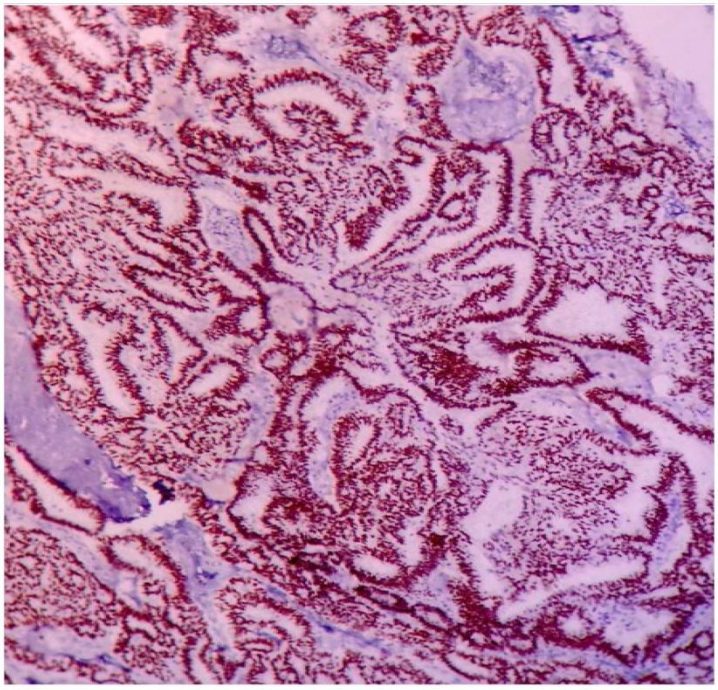


Figure.18 : ENDOMETRIOID CARCINOMA SHOWING STRONG ER EXPRESSION, NUCLEAR POSITIVITY (10X)

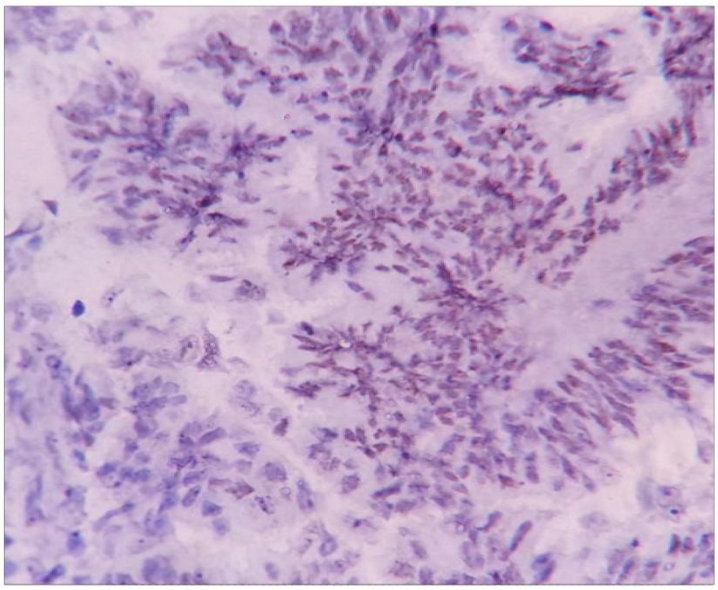


Figure.19 : ER EXPRESSION SHOWING FOCAL POSITIVITY (40X)

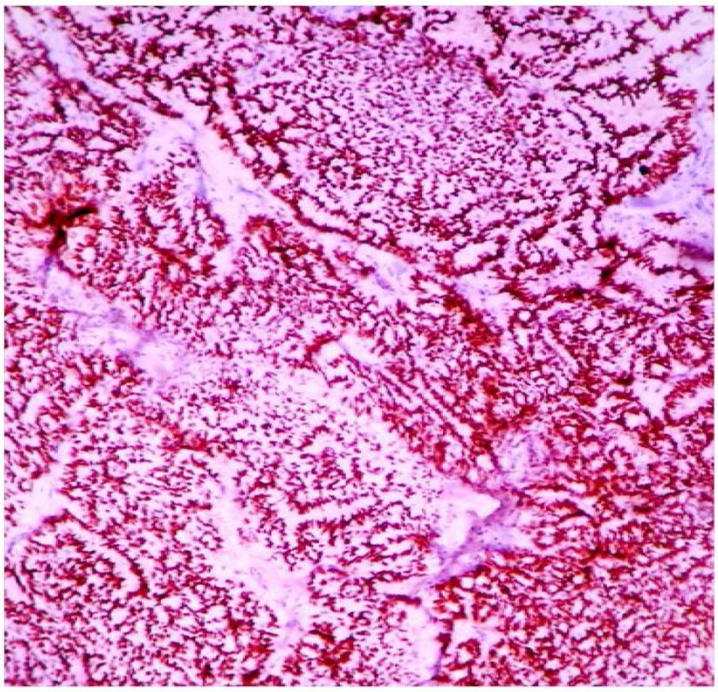


Figure.20 : ENDOMETRIOID CARCINOMA SHOWING STRONG PR EXPRESSION, NUCLEAR POSITIVITY (10X)

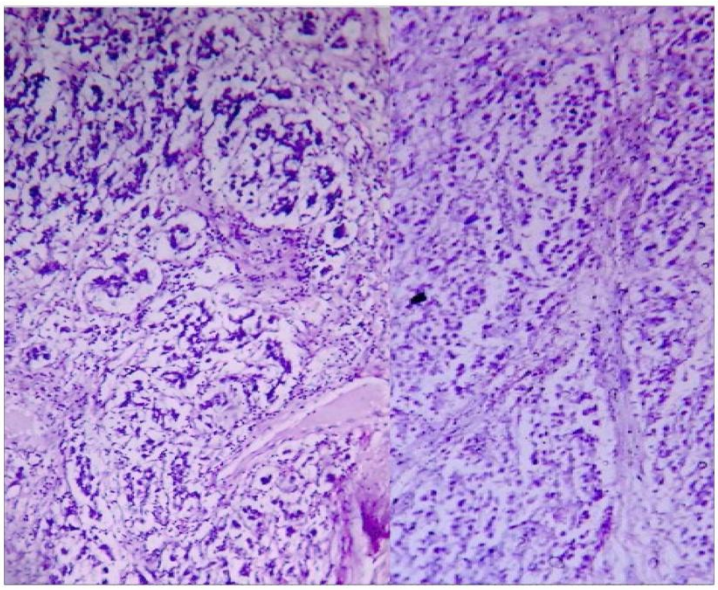


FIGURE.21 : MIXED GERM CELL TUMOUR (DYSGERMINOMA, EMBRYONAL CARCINOMA)

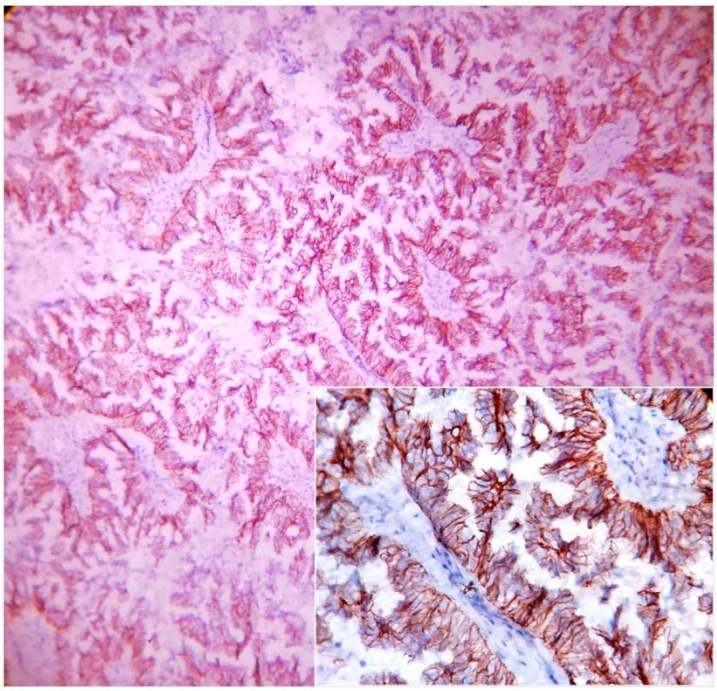


Figure.22 : HER-2/neu STRONG 3+ MEMBRANOUS POSITIVITY IN A HIGH GRADE SEROUS CARCINOMA

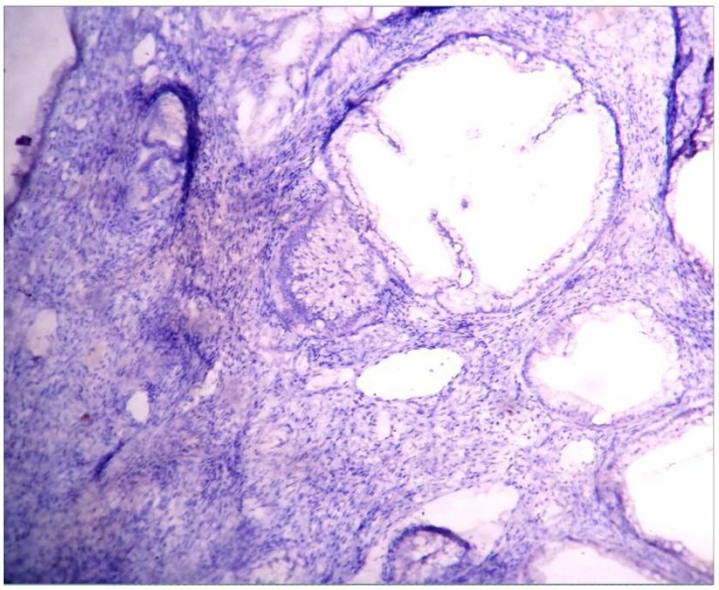


Figure.23 : HER-2/neu EXPRESSION NEGATIVE IN A SEROUS BORDERLINE (10X)

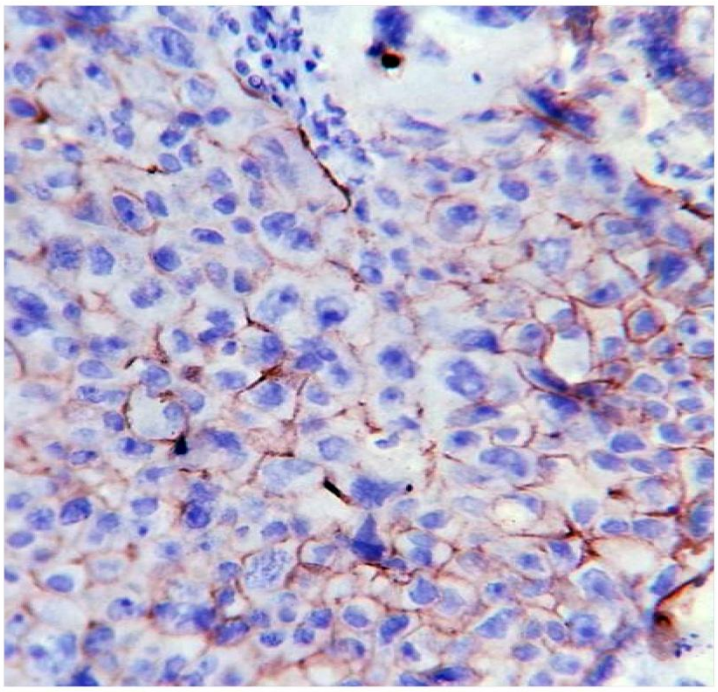


Figure.24 : HER-2/neu 2+ MEMBRANOUS POSITIVITY IN A HIGH GRADE CARCINOMA

DISCUSSION

Ovaries are common sites of non-neoplastic and neoplastic lesions. The constant cyclical changes from puberty to menopause give rise to different cell types, each of which results in a varieties of tumours[32].

The diverse histopathologies in ovarian tumours reflects the different cell origins[63]. Recent surveillance, epidemiology and end result (SEER) calculations of lifetime risk for ovarian carcinoma are that, 1 in 55 women will develop ovarian cancer over their life time[62]. As the symptoms are vague and manifest over time ovarian cancers are difficult to detect until they are in advanced stage[62].

Identification of various histological patterns of ovarian tumours is important in diagnosis, prognosis and treatment of ovarian cancers. Immunohistochemistry is now emerging as an important tool in diagnosis of ovarian tumours[10]. It has been shown that estrogen and progesterone receptor level depends on histological grade, stage and variables among the tumours of same grade[56]. Her-2/neu is amplified and overexpressed in 25 to 30% of human ovarian cancers. It is associated with progression of invasive cancer, poor prognosis and resistance to chemotherapy[53].

In this study, about 14,093 surgical specimens were received over a period of 2 ½ years. Of the 631 gynaecological tumours 132 ovarian neoplasms were reported. This accounts for about 20.9% of all gynaecological tumours reported in this study. Total number of malignancies reported during the study period is 1089 and total ovarian malignancies is 26.

I. INCIDENCE

In our study, the ovarian cancer constitute 2.1% of all female malignancies [Table 20, Chart 12].

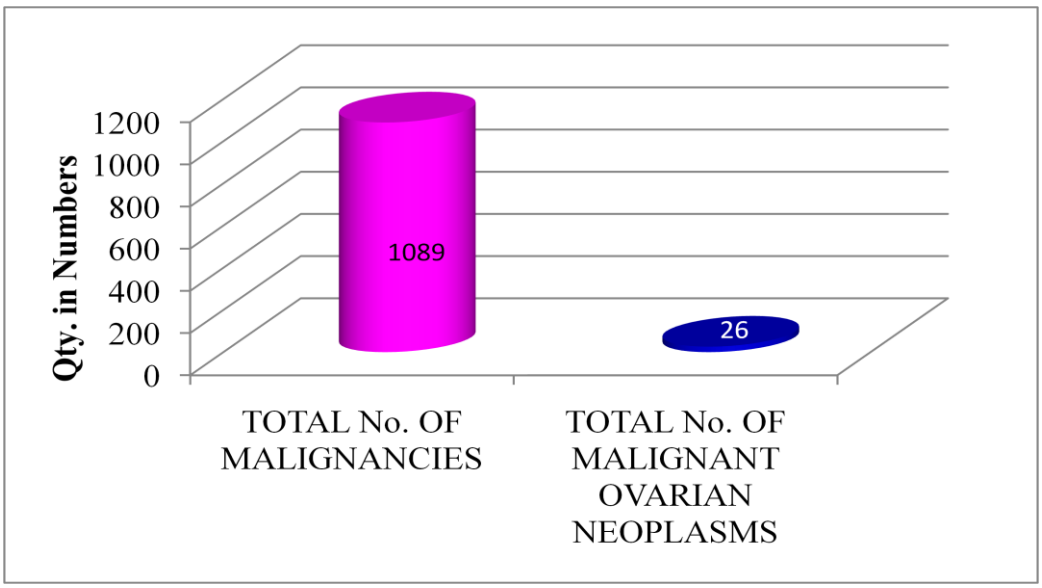
INCIDENCE OF MALIGNANT OVARIAN TUMOURS IN RELATION TO TOTAL MALIGNANCIES IN FEMALES.

TABLE 20:

Sl. No.	PERIOD	TOTAL No. OF MALIGNANCIES IN FEMALES	TOTAL No. OF MALIGNANT OVARIAN NEOPLASMS	PERCENTAGE
1	Jan. 13 to May 13	274	4	0.3%
2	June 13 to Dec. 13	244	7	0.6%
3	Jan. 14 to May 14	192	6	0.5%
4	June 14 to Dec. 14.	269	4	0.3%
5	Jan. 15 to May 15	110	5	0.4%
	Total	**1089**	**26**	**2.1%**

In our study period spanning for a period of 2 ½ years, a total of 1089 female malignancies were reported. Out of which 26 ovarian malignancies were reported. This accounts for about 2.1%. The above is depicted in Chart 12.

CHART 12: INCIDENCE OF MALIGNANT OVARIAN TUMOURS IN RELATION TO TOTAL FEMALE MALIGNANCY



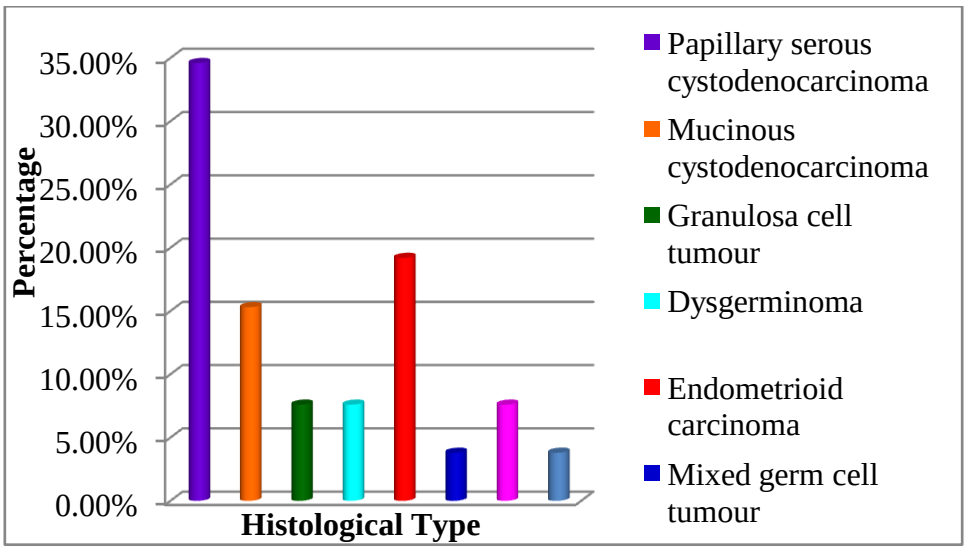
II. INCIDENCE OF HISTOLOGICAL TYPES OF MALIGNANT TUMOURS IN RELATION TO TOTAL OVARIAN MALIGNANCIES.

TABLE 21:

HISTOLOGICAL TYPE	No. OF CASES	% OF TOTAL MALIGNANT OVARIAN TUMOUR(26)	% INCIDENCE OF TOTAL OVARIAN TUMOURS (132)
Papillary serous cystadenocarcinoma	9	34.6%	6.8%
Mucinous cystadenocarcinoma	4	15.3%	3.0%
Granulosa cell tumour	2	7.6%	1.5%
Dysgerminoma	2	7.6%	1.5%
Endometrioid carcinoma	5	19.2%	3.7%
Mixed germ cell tumour	1	3.8%	0.7%
Metastatic adeno/krukenberg's	2	7.6%	1.5%
Immature teratoma	1	3.8%	0.7%

From the above Table 21 it is inferred that of total 26 malignant ovarian tumours most common was papillary serous cystadenocarcinoma. The above equates to 34.6% (9/26) followed by Endometrioid carcinoma accounting for 19.2% (5/26). The least common type is mixed germ cell tumour and immature teratoma (Chart 13).

CHART 13: INCIDENCE OF MALIGNANT OVARIAN TUMOURS



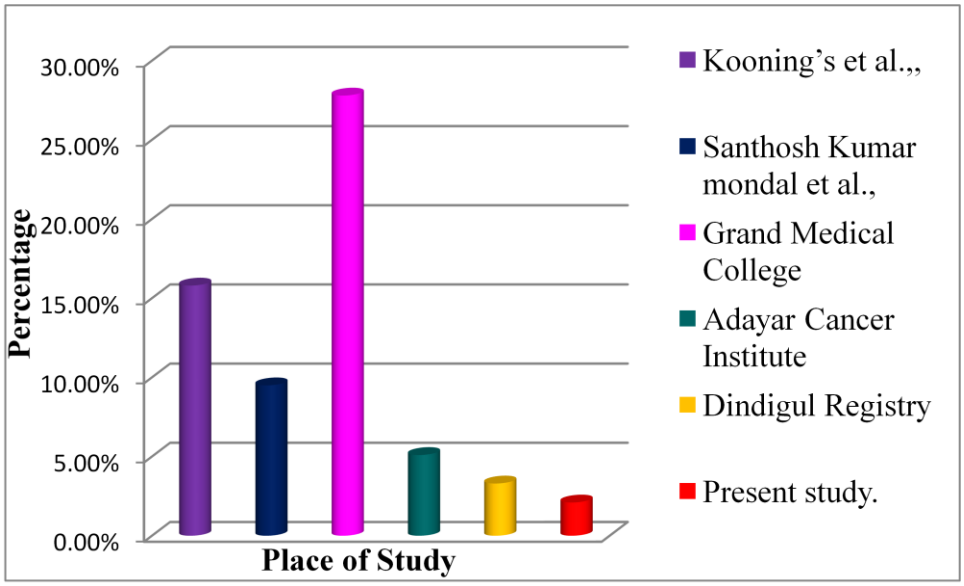
III. INCIDENCE OF OVARIAN CARCINOMA IN RELATION TO OTHER STUDIES:

TABLE-22:

S.NO.	PLACE OF STUDY	INCIDENCE
1.	Kooning's *et al.*,, United States	15.8%
2.	Santhosh Kumar mondal *et al.*, [2011] in Eastern India	9.5%
3.	Grand Medical College and Sir J.J. group of Hospitals., Mumbai.	27.8%
4.	Adayar Cancer Institute, Chennai.	5.1%
5.	Dindigul Ambilikkai Cancer registry	3.3%
6.	Present study.	2.1%

From the above table it is evident, the incidence rate is highest (15.8%) in developed countries. In India, the incidence rate is highest in Mumbai (27.8%) and least is Dindigul Ambilikkai Cancer Registry (3.3%). Our study is undertaken in a semiurban area with an incidence rate of 2.1%. This is midway between a rural and urban area [Chart-14]

CHART 14: INCIDENCE OF OVARIAN CARCINOMA IN RELATION TO OTHER STUDIES



IV. INCIDENCE OF OVARIAN MALIGNANCIES IN COMPARISON WITH OTHER FEMALE GENITAL TRACT MALIGNANCIES:

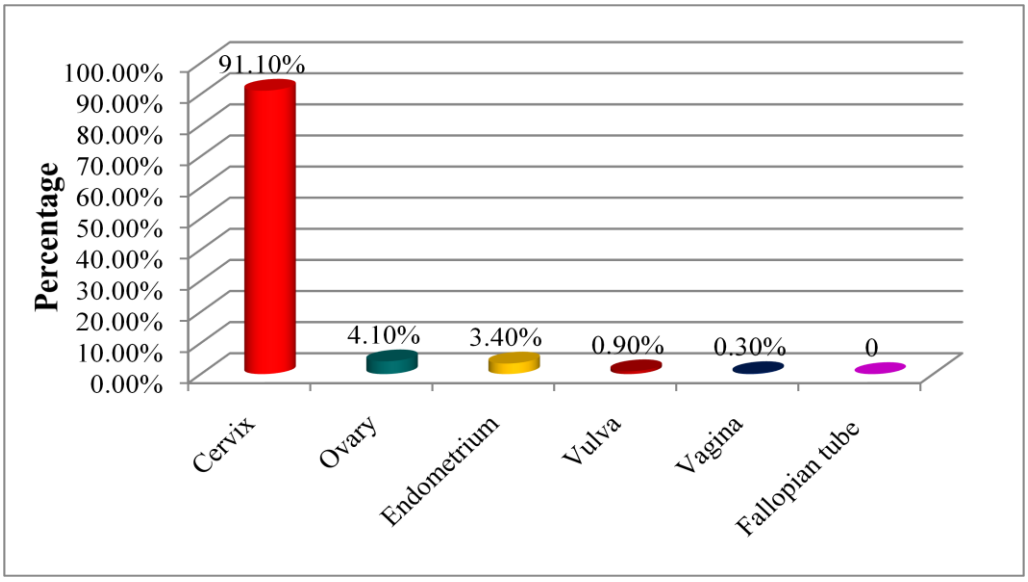
TABLE-23.

S.NO.	SITE	NO. OF FEMALE GENITAL TRACT MALIGNANCIES	PERCENTAGE
1.	Cervix	578	91.1%
2.	Ovary	26	4.1%
3.	Endometrium	22	3.4%
4.	Vulva	6	0.9%
5.	Vagina	2	0.3%
6.	Fallopian tube	-	-
	Total	**634**	

From the above table, we infer that ovarian cancers are the second most common (4.1%) malignancy among all female genital tract malignancies. Uterine cervix (91.1%) is the most common site to be involved and the least common site is vagina (Chart 15).

The age specific incidence of ovarian neoplasm ranges from 20-70 years. In America, Koonings *et al.,* reported 94% of benign tumours were common in the age group of 20-29 years and 92% of the malignant tumours were common after sixth decade.[22] In our study, 27.8% of Benign neoplasm were common in the age group of 20-29 years and the malignant tumour were common in 5th decade. This study correlates well with studies conducted by Santhosh Kumar mondal *et al.,*[63] Kayastha *et al.,*[64]. In comparison with western countries, our study proves, an earlier age of onset of malignant tumours.

CHART 15: INCIDENCE OF OVARIAN MALIGNANCIES IN RELATION TO FEMALE GENITAL TRACT MALIGNANCIES



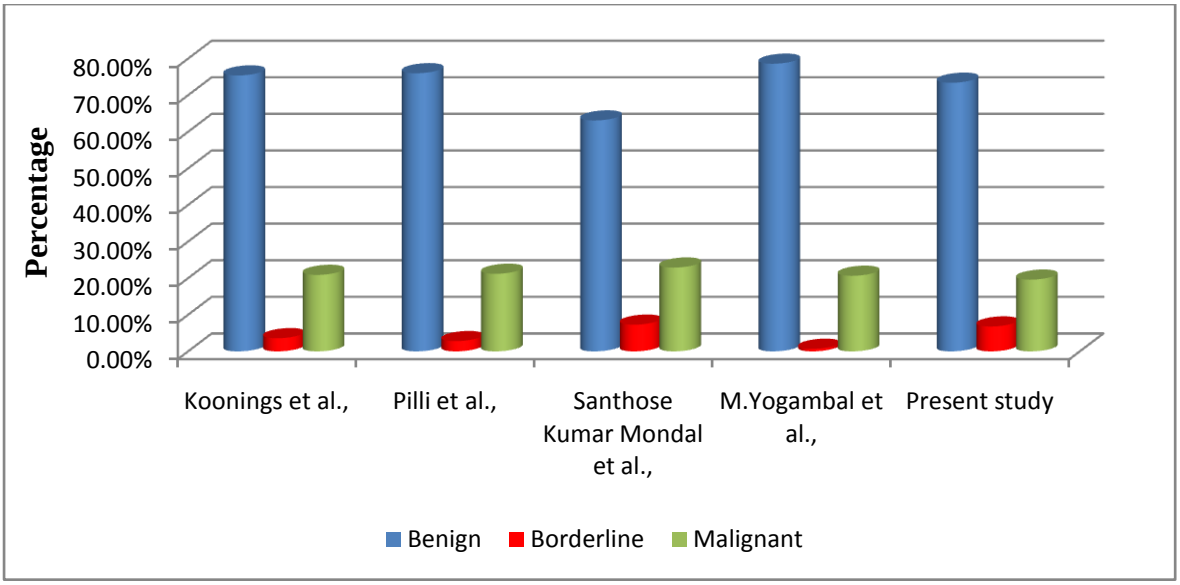
V. COMPARISON OF PERCENTAGE OF BENIGN, BORDERLINE AND MALIGNANT TUMOURS WITH OTHER STUDIES:

TABLE-24:

S.NO.	AUTHOR KOONINGS *et al.,*	BENIGN	BORDER LINE	MALIGNANT
1.	Koonings *et al.,*	75.4%	3.6%	20.9%
2.	Pilli *et al.,*	76%	2.8%	21.2%
3.	Santhosh Kumar Mondal *et al.,*	63.1%	7.3%	22.9%
4.	M.Yogambal *et al.,*	78.6%	0.7%	20.65%
5.	Present study	73.4%	6.8%	19.6%

The results of our study is comparable with Koonings *et al.,*[22], Pilli *et al.,*[66], and M.Yogambal *et al.,*[65]. Comparison of incidence of benign, borderline and malignant tumours with other in our studies is depicted in table 24 (Chart-16).

CHART 16: INCIDENCE OF OVARIAN TUMOURS IN RELATION TO OTHER STUDIES



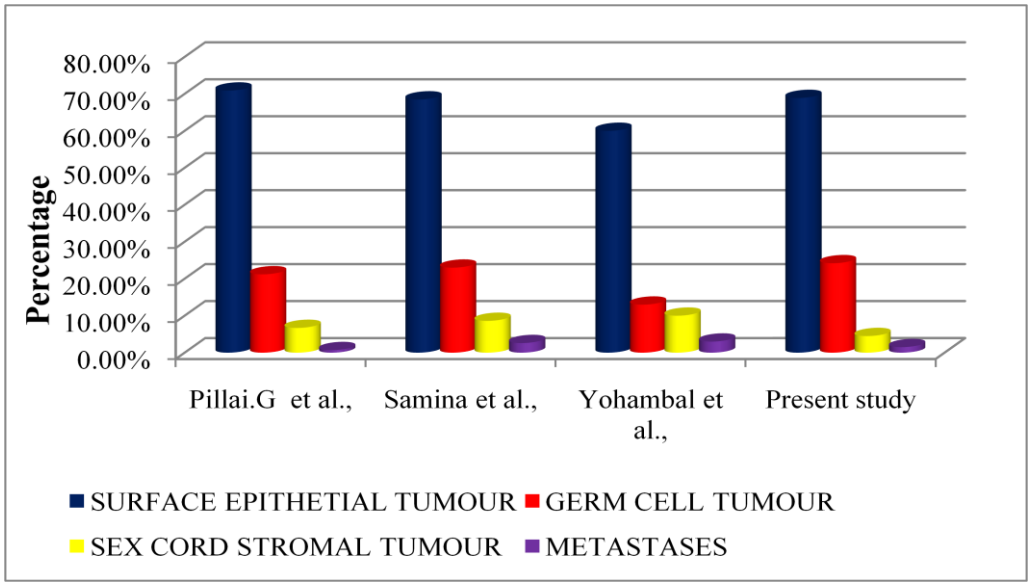
VI. COMPARISON OF INCIDENCE OF HISTOLOGICAL TYPES OF OVARIAN TUMOURS WITH OTHER STUDIES.

TABLE-25:

S.NO.	AUTHOR	PLACE OF STUDY	SURFACE EPITHELIAL TUMOUR	GERM CELL TUMOUR	SEX CORD STROMAL TUMOUR	METASTASES
1.	Pillai.G *et al.,*	India	70.9%	21.2%	6.7%	0.7%
2.	Samina *et al.,*	Pakistan	68.5%	23%	8.6%	2.6%
3.	Yohambal *et al.,*	Chennai	60%	13%	10%	3%
4.	Present study	Thanjavur	68.9%	24.2%	4.5%	1.5%

In our study, surface epithelial tumours constitute the majority of ovarian tumour (68.9%) followed by germ cell tumours (24.2%) and sex cord stromal tumours (4.5%). The above data coincides with Samina *et al.,*[67] in which surface epithelial tumour constituted 68.5% and germ cell tumour (23%). The above table compares the incidence of histological types of ovarian tumours with other studies Table-25 (Chart-17).

CHART 17: COMPARING THE INCIDENCE OF HISTOLOGICAL TYPES OF OVARIAN TUMOURS IN RELATION TO OTHER STUDIES



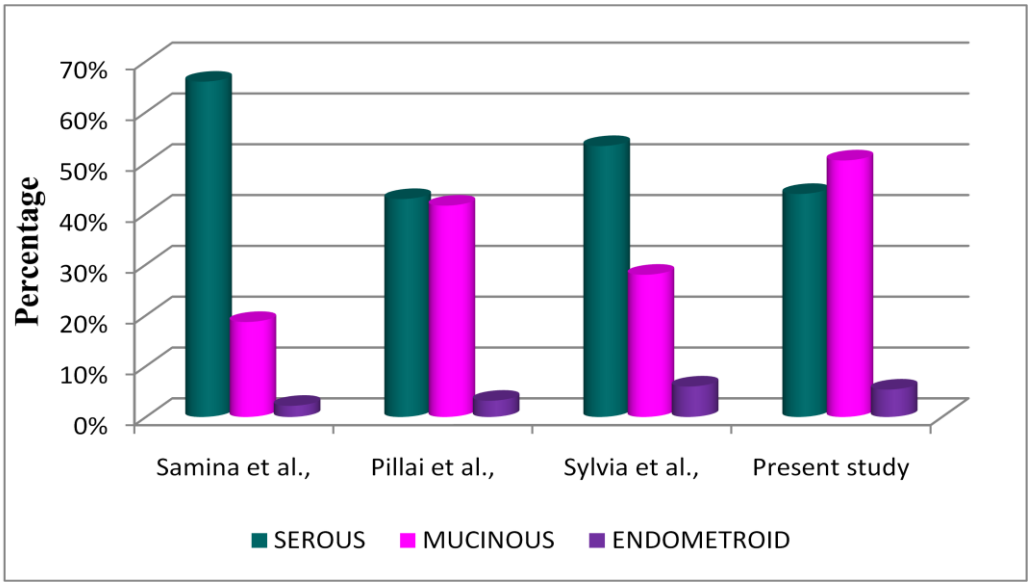
VII. COMPARISON OF INCIDENCE OF HISTOLOGICAL SUB TYPES OF SURFACE EPITHELIAL TUMOURS IN RELATION TO OTHER STUDIES.

TABLE-26:

S.NO.	AUTHOR	PLACE OF STUDY	SEROUS	MUCINOUS	ENDOMETRIOID
1.	Samina *et al.,*	Pakistan	66%	18.7%	2.2%
2.	Pillai *et al.,*	India	42.9%	41.6%	3.2%
3.	Sylvia *et al.,*	Pondicherry	53.33%	28%	6%
4.	Present study	Thanjavur	43.9%	50.5%	5.4%

Among all surface epithelial ovarian tumours, mucinous tumours (41.6%) constitute the commonest followed by serous (43.9%) and Endometrioid (5.4%). This is not in concordance with the other three studies mentioned above. The above table 26 compares the incidence of histological types of surface epithelial tumours in relation to other studies (Chart-18)

CHART 18: COMPARING THE INCIDENCE OF HISTOLOGICAL SUB TYPES OF SURFACE EPITHILIAL TUMOURS IN RELATION TO OTHER STUDIES



VIII. COMPARISON OF SEROUS TUMOURS INCIDENCE OTHER STUDIES:

TABLE-27:

S.NO.	AUTHOR	PLACE OF STUDY	BENIGN	BORDERLINE	MALIGNANT
1.	Misra *et al.,*	Uttar Pradesh	75.2%	2.3%	21.1%
2.	Samina *et al.,*	Pakistan	38%	2%	4.9%
3.	Hiremath *et al.,*	Pondicherry	46%	8%	13.5%
4.	Present study	Thanjavur	70%	7.5%	22.5%

According Jefferey *et al.,*[34], serous borderline tumors presented as commonest type among the borderline tumours. In contrast, our study depicts a higher incidence of mucinous borderline tumours (66.6%) when compared to serous borderline tumours (33.3%).

Neeraj lalwani *et al.,*[30] in his study states 1/3rd of serous borderline tumours were bilateral and were associated with peritoneal implants . In contrast in our study all the serous borderline tumours were unilateral and none had positive peritoneal implants.

According to Anais malpica *et al.,*[52], ovarian serous tumours are divided into low grade and high grade according to recent two tier grading system. The above phenomena is based primarily on nuclear atypia and the secondary feature , mitotic rate.

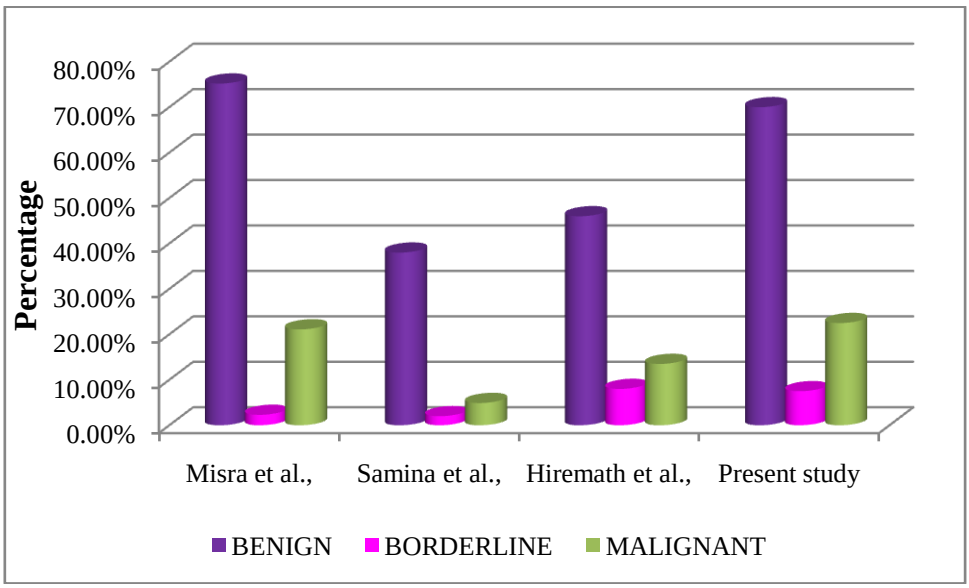
According to Jefferay SD *et al.,*[34] surface epithelial carcinomas are divided into Type I and II based on different pathways of tumorigenesis

According to Robert J.Kurman the low grade neoplasm arise through Stepwise process from borderline tumours. These include low grade serous, mucinous, Endometrioid, malignant Brenner, clear cell Carcinomas [21]. Type II tumours arising denovo are aggressive

neoplasms. Type II tumours present at advanced stages. These include high grade serous, malignant mixed mullerian tumour, undifferentiated carcinoma.[52,55]

The incidence of Benign Borderline and malignant tumours in our study is compared with other studies (Table-27) and is represented in Chart-19.

CHART 19: COMPARING THE INCIDENCE OF SEROUS TUMOURS IN RELATION TO OTHER STUDIES



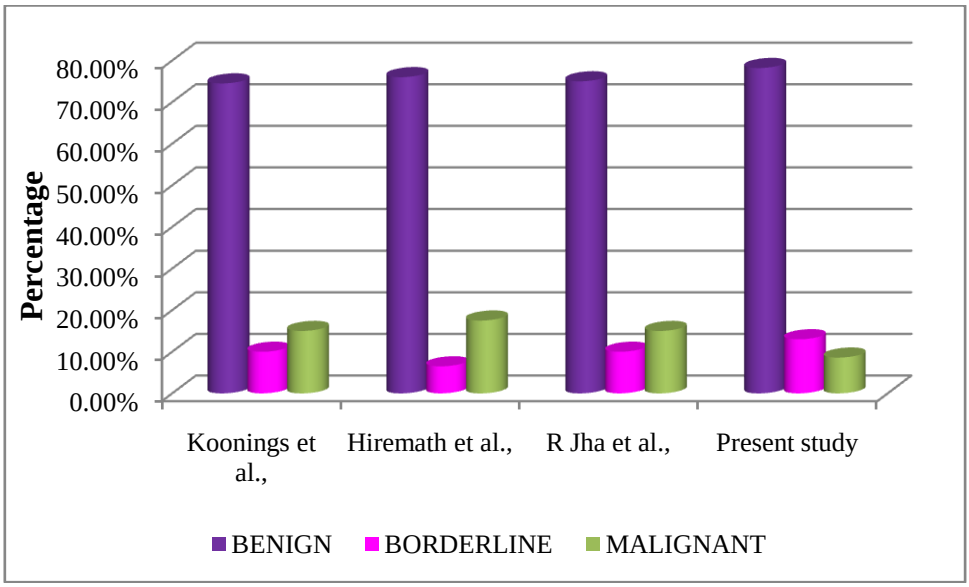
IX. COMPARISON OF MUCINOUS TUMOURS INCIDENCE WITH OTHER STUDIES

TABLE-28

S.NO.	AUTHOR	PLACE OF STUDY	BENIGN	BORDERLINE	MALIGNANT
1.	Koonings *et al.,*	USA	74.5%	10%	15%
2.	Hiremath *et al.,*	Pondicherry	76%	6.5%	17.5%
3.	R Jha *et al.,*	Nepal	75%	10%	15%
4.	Present study	Thanjavur	78.2%	13%	8.6%

In our study mucinous tumour is the most common surface epithelial tumour of which 78.2% were benign, 13% were borderline and 8.6% were malignant. This is in contrast to the studies of Koonings *et al.,*[22] Hiremath *et al.,*[62] Misra *et al.,*[68] which stated that serous tumours constituted the commonest type of surface epithelial tumours. The above (Table 28) compares the incidence of mucinous tumour in our institution with respect to other studies and is also depicted in the (Chart-20)

CHART 20: COMPARING THE INCIDENCE OF MUCINOUS TUMOURS IN RELATION TO OTHER STUDIES



According to Jaime *et al.,*[60] endometriosis is associated with high incidence of Endometrioid, clear cell carcinomas, mucinous and serous carcinoma. In contrast in our study none of Endometrioid carcinoma (5/132) cases were associated with endometriosis.

In our study 33 cases of germ cell tumours were reported. This constitute about 24.2% of all ovarian neoplasms. In germ cell tumours, mature cystic teratoma is the commonest group constitutes 90.9%, followed by dysgerminoma 6% and mixed germ cell tumour 3%. This is in concordance with studies of Kwok *et al.,*[43] and Norries HJ *et al.,*[44] . According to the above authors, malignancy in germ cell tumours is associated with post menopausal age group. This is in contrast to our study where one case which was reported as immature teratoma, was reported in younger age group.

According to P.Singh *et al.,*[47] the commonest tumour associated with pregnancy is dermoid cyst. In our study ,2 cases were reported which were associated with pregnancy. One was associated with dermoid cyst, the other case was reported in association with mucinous cyst adenoma. One case of mixed germ cell tumour was reported. This tumour was a combination of Dysgerminoma and Embryonal carcinoma.

In our study, a total of 6 cases of sex cord stromal tumours were reported . This accounts for about 4.5% of all ovarian tumours. Granulosa cell tumour, fibroma, fibrothecoma had the same incidence of 33.3%. The above data had no coincidence with any of the studies.

According to Lawrence *et al.,*[69] 95% of granulosa cell tumours are unilateral. All the 2 cases in our study were unilateral and none were associated with endometrial hyperplasia.

According to masaki mandai *et al.,*[50] 5-10% of neoplasm arise from metastases. Ovarian metastases have some common features. Some of them are bilaterality, surface involvement, extensive extra ovarian spread, desmoplastic reaction, vascular invasion and

unusual clinical history[16,50]. In our study one case of Krukenberg's and one case of metastatic adeno carcinomatous deposit was reported. Both were unilateral in presentation.

The krukenberg's tumour was characterised by the presence of mucin filled, signet ring tumour cells within cellular stroma.

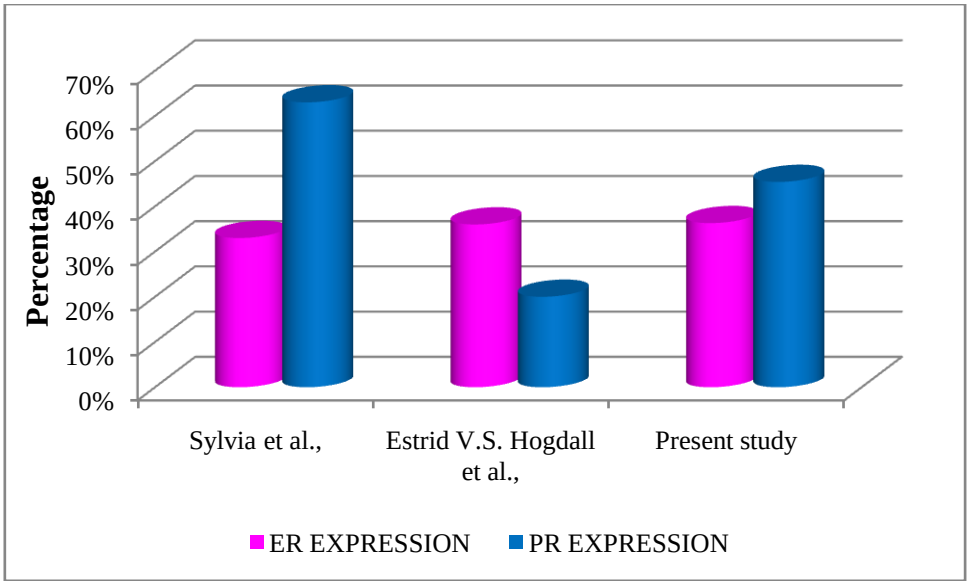
X. COMPARISON OF INCIDENCE OF ER/PR EXPRESSION IN SURFACE EPITHELIAL TUMOURS. IN RELATION TO OTHER STUDIES:

TABLE-29:

S.NO.	AUTHOR	ER EXPRESSION	PR EXPRESSION
1.	Sylvia *et al.,*	33%	63%
2.	Estrid V.S. Hogdall *et al.,*	36%	20%
3.	Present study	36.3%	45.4%

In our study, ER expression is positive in 4/11 cases ((36.3%) cases, PR expression is positive in 5/11 (45.4%) which coincides with study of Sylvia *et al.,*[26]. ER,PR has strong expression in Endometrioid (2/3,66.6%) and high grade serous tumours (3/5 cases 80%) which is similar to findings of Agarwal *et al.,*[70], Damia *et al.,*[77]. This is in contrast to Buchynska *et al.,*[72] who stated that ER expression is lower in high grade tumour. The above features suggests possible mitogenic role of ER in ovarian tumours and high PR positivity in conjunction with ER expression is indicative of estrogen regulated disease. The above is depicted in (Chart-21).

CHART 21: INCIDENCE OF ER, PR EXPRESSION IN SURFACE EPITHELIAL TUMOURS IN RELATION TO OTHER STUDIES



XI. COMPARISON OF EXPRESSION OF Her-2/neu IN BORDERLINE AND MALIGNANT SURFACE EPITHELIAL TUMOURS.

TABLE-30:

S.NO.	AUTHOR	BORDERLINE	MALIGNANT
1.	Sapna Go *et al.,*	-	48.6%
2.	Sylvia *et al.,*	1%	21%
3.	Present study	-	36.3%

From the above table, we infer that HER-2 expression is negative in borderline tumours and shows positivity in high grade malignant tumours mostly commonly in high grade serous tumours (3/5 cases, 60%). This is in concordance with Sylvia *et al.,*[26] and Sapna Go *et al.,*[74].

Borderline tumours occur in younger women less than 40 years of age. To preserve fertility, conservative surgery is the treatment of choice. These patients should be monitored routinely for CA-125 serum levels and ultrasound examinations. The clinical course lies in between the benign and malignant tumours. Evidence suggests they are known to metastasize within the peritoneal cavity[28].

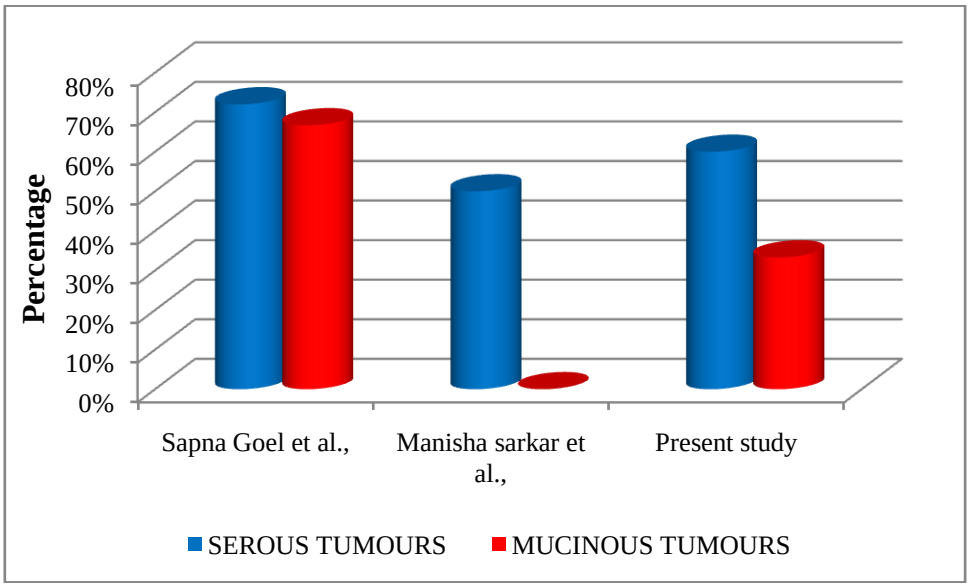
In mucinous borderline tumours the identification of stromal invasion is difficult. This is because of gross and microscopic multiloculation. But, atypical nuclei and stratification of more than 3 layers suggests well differentiated mucinous carcinoma even when there is no stromal invasion[28].

In our study, Her-2/neu was positive (3/5 cases 60%) in serous tumours and 1/3 (33.3%) cases of mucinous tumours. This is in concordance with studies of sapna Go *et al.*,[74] and is depicted in the following Table-31 and Chart-22.

Table 31:

S.NO.	AUTHOR	EXPRESSION OF Her-2/neu IN	
		SEROUS TUMOURS	MUCINOUS TUMOURS
1.	Sapna Goel *et al.*,	72%	66.7%
2.	Manisha sarkar *et al.*,	50%	-
3.	Present study	60%	33.3%

CHART 22: COMPARING THE EXPRESSION OF Her-2/neu IN RELATION TO OTHER STUDIES



The scoring system of Her-2/neu, showed variations, in individual authors study which is depicted in the following Table32.

Table 32:

S.NO.	AUTHOR	SCORING OF Her-2/neu IN SURFACE EPITHELIAL TUMOURS	
		3+	**1+,2+**
1.	Manisha Sarkar *et al.*,	35%	20%
2.	Marvann *et al.*,	6%	32%
3.	Present study	27%	36.3%

In our study, the intensity of positivity did not correlate with age, size and grade of the tumours. This is in concordance with Sapna Goel *et al.*,[74] and Sylvia *et al.*,[26] (Chart-22)

From the above data, Her-2/neu expression is found to be positive only in malignant tumours. This suggests their carcinogenic role and helps in distinguishing borderline and malignant tumours.

CONCLUSION:

A total of 132 cases were evaluated in concordance with clinical history, histopathological and immunohistochemistry and the following conclusions were arrived at.

1. The incidence of ovarian neoplasms among all female neoplasms is 4.7%
2. The incidence of ovarian malignancies among all female malignancies is 2.1%
3. Ovarian malignancy (4.1%) is the second most common malignancy in female genital tract next to cervical malignancy.
4. In age groups, ovarian neoplasms are commoner in 2^{nd} decade and in 4^{th} decade.
5. The ratio of benign and malignant ovarian neoplasm is 3:1.
6. Predominantly they presented as unilateral tumours (90%) than bilateral (10%).
7. Grossly, benign tumours presented, as cystic neoplasms and the malignant tumours presented as solid and cystic or purely solid.
8. Regarding Histological type, surface epithelial tumours (68.9%) are the most common neoplasm among which mucinous cystadenoma is the commonest.
9. Positive expression of ER,PR (steroid receptors) in surface epithelial malignancies proves the mitogenic role of estrogen in ovarian tumours. PR expression may be related to estrogenic regulation. The expression of steroid receptors paves way for antihormonal therapy.
10. Her-2 neu was expressed only in malignant tumours. This suggests their carcinogenic role. This also helps in differentiating borderline and malignant tumours.

This study is an institution based one with a small sample size of 132 cases. The results may not actually reflect the original age distribution and histological pattern of ovarian tumours in Indian population. The epidemiological data of developed countries in many aspects differ from the developing nations. The differences about hormone receptors expression by different authors may be due to various parameters like case selection, method

of immunohistochemistry and sample size and different grading systems. This should be standardized to identify reliable prognostic markers in the clinical trial of hormone therapy. A multicentric, large population based study with facility of follow up will be needed to prove the prognostic significance of anti Her-2/neu therapy in surface epithelial carcinomas. Thus a panel of markers will be helpful in prognostication of ovarian tumour and development of targeted therapy. This study will serve as a reference for future studies. Continued research studies in future would accomplish an effective therapy for ovarian cancer.

APPENDIX I

HAEMATOXYLIN AND EOSIN STAIN

Preparation of solution :

HARRIS HAEMATOXYLIN

Distilled water-1000ml

Ammonium alum-100g

Absolute ethyl alcohol-50ml

Mercuric oxide-2.5g

100g of ammonium alum dissolved in 1000ml of distilled water by heating and shaking at 60.c. Add solution of 5g of haematoxylin in 50ml of ethylalcohol and bring rapidly to boil. when it begins to boil, remove from flame and add 2.5 g of mercuric oxide. Mix by swirling gently.

EOSIN STAIN

Eosin Y- 1 g.

Distilled water-20ml.

95% ethanol-80ml

Glacial acetic acid-0.2ml

Dissolve 1 gm of eosin Y in 20ml of water, add 80 ml of 95% ethyl alcohol and 0.2 ml of glacial acetic acid.

Procedure :

1. Bring the sections to water
2. Dip in Harris haematoxylin for 15 minutes.
3. Rinse in tap water.
4. Differentiate in 1% acid alcohol-3-4 quick dips.
5. Wash in tap water briefly.
6. Dip in ammonia water or saturated lithium carbonate until the sections are blue.
7. Wash in running tap water for 10-20 minutes.
8. Stain with eosin for 15 seconds to 2 minutes depending on the age of the eosin and the depth of counter stain.
9. Rinse in tap water.
10. Dip in 95% alcohol
11. 3 changes in absolute alcohol.
12. Xylene – 2 changes.
13. Mount in DPX mountant.

APPENDIX II

IMMUNOHISTOCHEMISTRY

PREPARATION OF SOLUTIONS:

Tris buffer saline (TBS): 0.005M

Distilled water-10 litres

Sodium chloride- 80gms

Tris (hydroxymethylamine)-6.05g

1 M HCl-44 ml.

Final pH is adjusted to 7.6 with either 1 M HCl or 0.2 M Tris solution.

CITRATE BUFFER SOLUTION

Trisodium citrate-2.94 gm

1 N HCl-5ml

Distilled water-1000 ml

Final pH is adjusted to 6.0 with 1 N HCl.

Preparation of gelatine coated slides:

Chrome alum-0.05gm

Gelatine-0.3 gm

Distilled water-100 ml.

Chrome alum is added to distilled water and then heated to 60.c.gelatin is added slowly to the heated distilled water. Glass slides are then dipped in this solution and dried overnight.

Antigen retrieval:

The slides are placed in citrate buffer in the coplin jar and capped. The jar is then heated in a 750 W domestic microwave oven for 15 minutes.

Procedure :

1. Dewax the section in xylene (1/2 hr, 2 changes) and bring section to distilled water.
2. Antigen retrievel using TBS by microwave oven heating
3. Cool to room temperature in running tap water for 20 minutes.
4. Bring the section to TBS for 5 minutes.
5. Drain and wipe off excess TBS around sections
6. Incubate in endogenous peroxidase blocking agent for 15-20 minutes.
7. Gently wash the slide in TBS for 5 minutes.
8. Wipe off excess fluid and incubate in power block for 15-20 minutes
9. Blot and dry excess power block.
10. Incubate in primary antibody for 60 minutes.
11. Repeat steps 4 and 5.
12. Incubate in superenhancer for 30 minutes.
13. Repeat steps 4 and 5.
14. Incubate in secondary antibody for 30 minutes.
15. Repeat steps 4 and 5
16. Incubate in DAB(Diamino Benzidine) substrate buffer for 2-10 minutes
17. Wash in distilled water counter stain with haematoxylin, clear in xylene and mount with DPX.

APPENDIX III

Tumor, Node, Metastasis (TNM) Staging Scheme for ovarian carcinomas:

PRIMARY TUMOR (T)

TNM Category	International Federation of Gynaecology and Obstetrics-Obstetrics-FIGO	
TX		Primary tumor cannot be assessed
T0		No evidence of primary tumor
T1	I	Tumor limited to ovaries (one or both)
T1a	1A	Tumor limited to one ovary; capsule intact, no tumor on surface or no malignant cells in ascites/ peritoneal washings.
T1b	IB	Tumor limited to both ovaries, capsule intact, no tumor on surface or no malignant cells in ascites/ peritoneal washings.
T1c	IC	Tumor limited to one or both ovaries with any of the following: capsule ruptured, tumor on ovarian surface, malignant cells in ascites or peritoneal washings.
T2	II	Tumor involves one or both ovaries with pelvic extension and/or implants
T2a	IIA	Extension and/or implants on uterus and / or tubes. No malignant cells in ascites or peritoneal washings
T2b	IIB	Extension to and/or peritoneal implants to other pelvic tissues. No malignant cells in ascites or peritoneal washings.
T2c	II C	Pelvic extension and/or implants with malignant cells in peritoneal washings or ascites.
T3 and/or N1	III	Tumor involves one or both ovaries with microscopically confirmed peritoneal metastasis outside the pelvis.

T3a	IIIA	Microscopic peritoneal metastasis beyond the pelvis (no macroscopic tumor).
T3b	III B	Macroscopic peritoneal metastasis beyond the pelvis < 2 cm in greatest dimension.
T3c	III C	Macroscopic peritoneal metastasis beyond the pelvis > 2 cm in greatest dimension.
M1	IV	Distant metastasis (excludes peritoneal metastasis)

REGIONAL LYMPHNODE (N):

NX	Regional lymph nodes cannot be assessed
N0	No regional lymphnode metastasis.
N1	Regional lymphnode metastasis.

DISTANT METASTASIS (M):

MX	Distant metastasis cannot be assessed
M0	No distant metastasis.
M1	Distant metastasis (excludes peritoneal metastasis).

STAGE GROUPING:

Stage IA: T1a N0 M0

Stage 1B: T1b N0 M0

Stage 1C: T1c N0 M0

Stage IIA: T2a N0 M0

Stage IIB: T2b N0 M0

Stage IIC: T2c N0 M0

Stage IIIA: T3a N0 M0

Stage IIIB: T3b N0 M0

Stage IIIC: T3c N0 M0

Any T N1 M0

Stage IV : Any T Any N M1.

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